Exploring the non-specific effects of already recommended vaccines: A case for method triangulation

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Disclaimer

- MD, PhD, DMSc, MAE, Epidemiologist
- Mother of two kids, who received all the vaccines in the Danish programme
- Spent part of the last 32 years in Africa, studying the overall health effects of vaccines
- Co-developer of the concept of nonspecific effects of vaccines with Peter Aaby
- No financial conflicts of interests





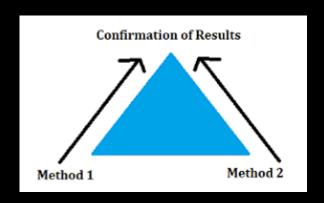


Two defining moments in my career

- Being a reviewer for the first time: Realizing I don't want to do "a Strachan"
- Working with an anthropologist:



> Triangulation



> Focus on what mattters:

Overall health outcomes (all-cause mortality and morbidity)

Påståeligt. Eksperter retter en hård kritik mod dansk vaccinepars forskning. Parret oversælger egne fund af ingenting, lyder anklagen. »Det er jo kejserens nye klæder,« siger professor.

En udødelig hypotese



The traditional understanding of vaccines

- A **vaccine** is a biological preparation that improves immunity to a particular disease
- Evaluation and monitoring is based on (biomarkers for) the vaccine disease and assessment of potential plausible adverse events
- None of the currently used vaccines were tested for their effect on the immune system's ability to handle other infections

Assumption: the immune system does not learn anything from meeting one pathogen that can be used in the meeting with other pathogens



Protective effect against vaccine disease

Adverse events

+/-

Non-specific effect on other diseases

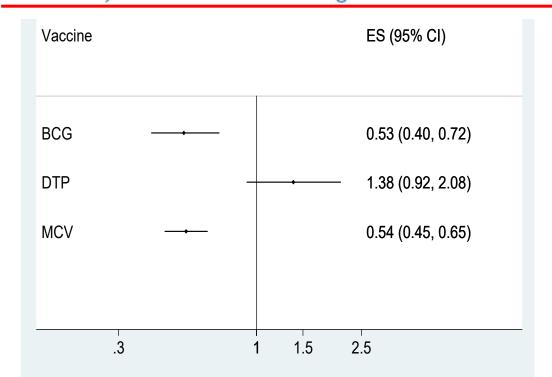
Effect on overall health

WHO review of non-specific effects

Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review

Julian P T Higgins,¹ Karla Soares-Weiser,² José A López-López,¹ Artemisia Kakourou,³
Katherine Chaplin,¹ Hannah Christensen,¹ Natasha K Martin,¹,⁴ Jonathan A C Sterne,¹
Arthur L Reingold⁵
the bmj | BMJ 2016;355:i5170 | doi: 10.1136/bmj.i5170

Receipt of BCG and measles containing vaccines may reduce overall mortality by
more than expected through their effects on the diseases they prevent, and receipt
of DTP may be associated with higher all cause mortality



Vaccinology: time to change the paradigm?

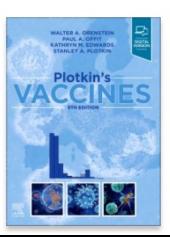
Christine Stabell Benn, Ane B Fisker, Andreas Rieckmann, Signe Sørup, Peter Aaby

Lancet Infect Dis 2020

Milestones | 28 September 2020

Nature Milestones in Vaccines





Plotkin's Vaccines

8th Edition - December 21, 2022

Authors: Walter A. Orenstein, Paul A. Offit, Kathryn M.

Edwards, Stanley A. Plotkin

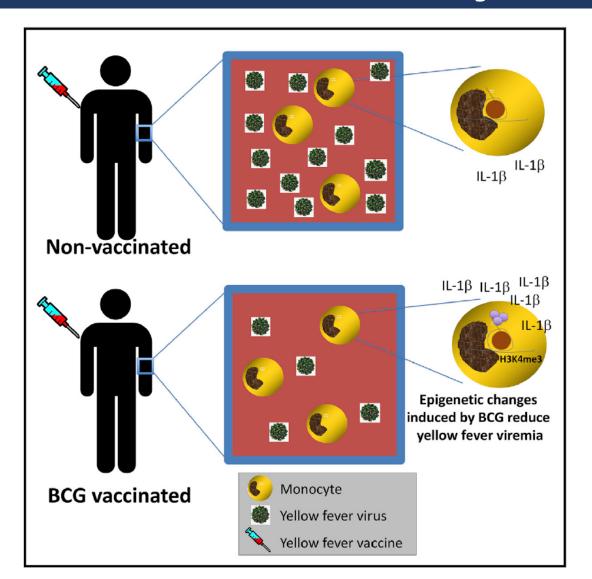
Language: English · Hardback ISBN: 9780323790581

eBook ISBN: 9780323790598

Chapter 3 Non-specific Effects of Vaccines

Immunological mechanisms: "Trained innate Immunity" Proof of principle:

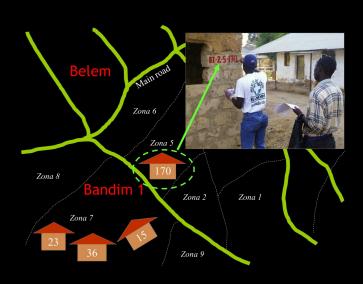
BCG 4 weeks prior to yellow fever vaccine reduces viremia in a human infectious challenge model





Bandim Health Project









Follow-up of a population ~200,000 individuals – personal identifier, censuses, home visits, registration of vaccines and other interventions, and of mortality and morbidity

Vaccinations

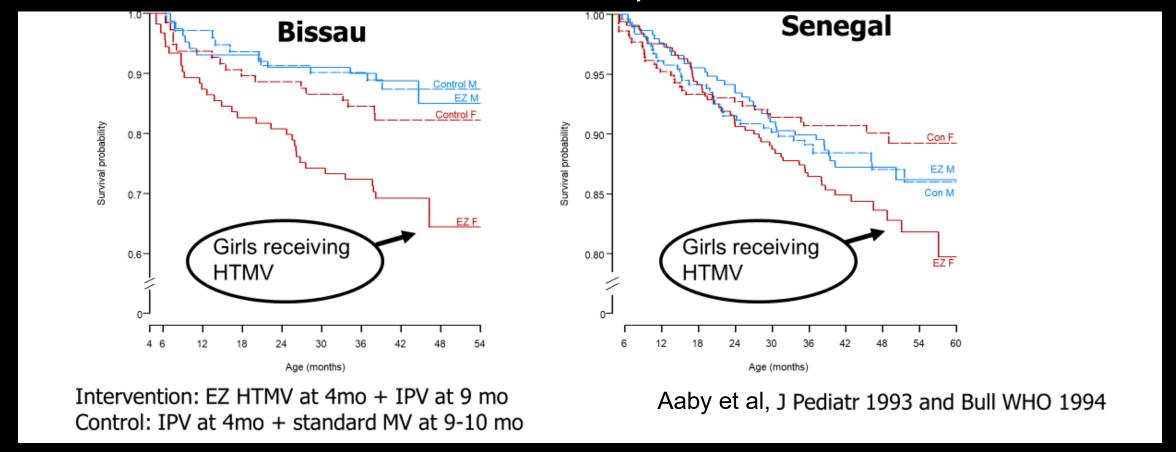
Effect on overall mortality and morbidity

An African field station (in collaboration between University of Southern Denmark and Guinea-Bissau)

The first RCTs showing non-specific effects

- High-titre measles vaccine (HTMV)
- Approved in low-income settings in 1989 could be given at 4-6 months of age, fully protective against measles infection
- Only studied for its effect on antibody and clinical measles (according to the traditional paradigm for vaccines)
- RCTs: Independent researchers tested the HTMV given at 4-6 months vs the standard measles vaccine given at 9 months

High-titre measles vaccine (HTMV) was associated with 2-fold higher all-cause mortality in females



When the same was found in Sudan and Haiti, WHO withdrew HTMV in 1992.

- Two fully protective vaccines had different effects on overall mortality.
- Specific-disease protection ≠ overall-benefit => Vacccines have non-specific effects (and they can be sex-differential)

Studying already recommended vaccines that cannot typically be studied in RCTs: Triangulation of data



International Journal of Epidemiology, 2016, 1866–1886

doi: 10.1093/ije/dyw314

Advance Access Publication Date: 20 January 2017

Original article

Approaches to causal inference

Triangulation in aetiological epidemiology

Debbie A Lawlor, 1,2,* Kate Tilling 1,2 and George Davey Smith 1,2

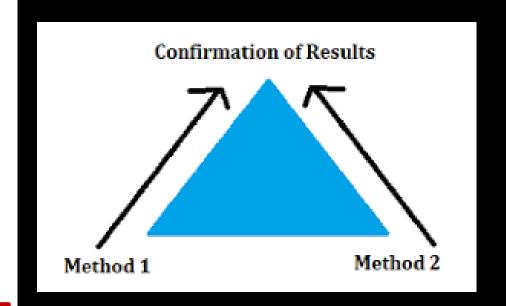
¹MRC Integrative Epidemiology Unit at the University of Bristol, Bristol, UK and ²School of Social and Community Medicine, University of Bristol, Bristol, UK

*Corresponding author. MRC IEU, University of Bristol, Oakfield House, Oakfield Grove, Bristol BS8 2BN, UK. E-mail: d.a.lawlor@bristol.ac.uk

Accepted 3 October 2016

Abstract

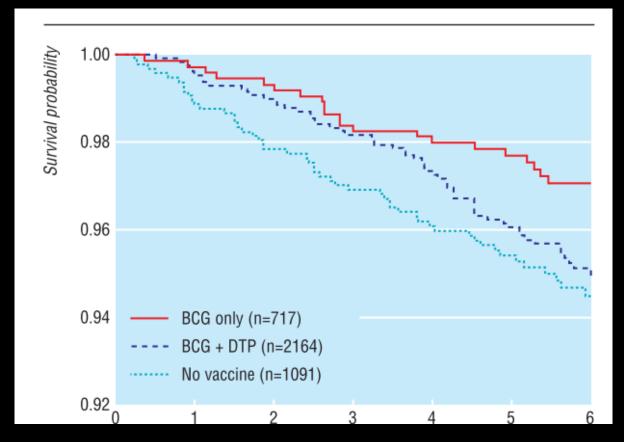
Triangulation is the practice of obtaining more reliable answers to research questions through integrating results from several different approaches, where each approach has different key sources of potential bias that are unrelated to each other. With respect to



An example of our research methodology: Neonatal Bacille Calmette-Guérin (BCG) vaccine



First of many observational studies of BCG vaccine



Kristensen et al, BMJ 2000

BCG: 45% (15-64%) reduction in all-cause mortality

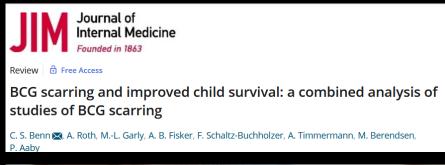
Could be healthy vaccinee bias, but

DTP: 84% (10-210%) increase in all-cause mortality

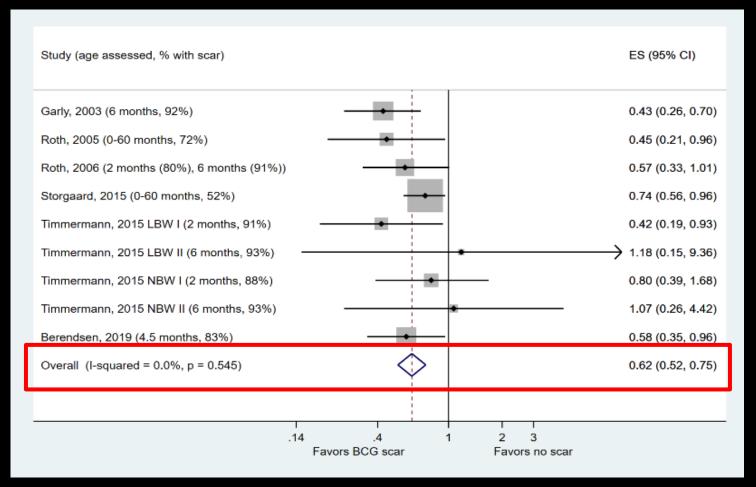
Divergent effect of different vaccines suggest there is more at play

Removing healthy vaccinee bias: Focusing on BCG-vaccinated children w/ and wo/ BCG scar

BCG scar: A marker of successful BCG vaccination. Finding 38% (25-48%) lower all-cause mortality among BCG-vaccinated children w/ scar vs among BCG vaccinated children wo/ scar supports a biological effect of BCG on all-cause mortality

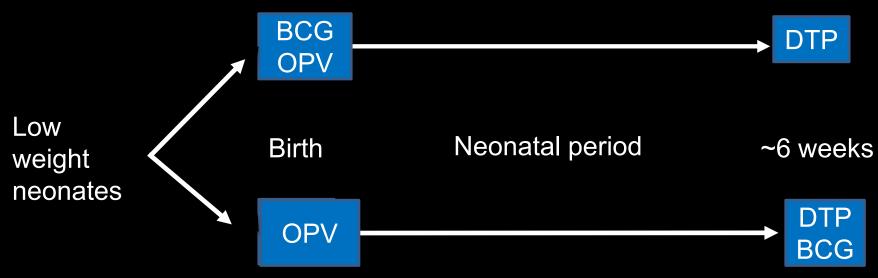




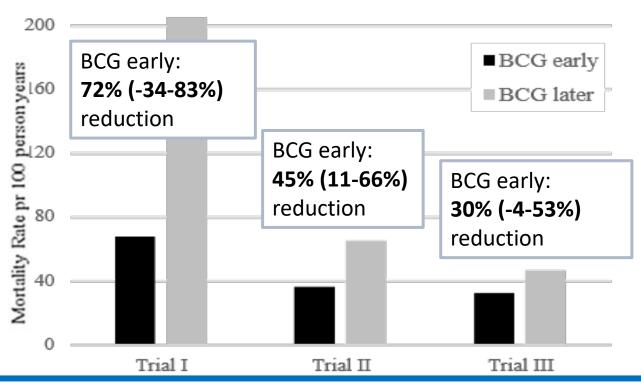


RCTs of BCG vaccine





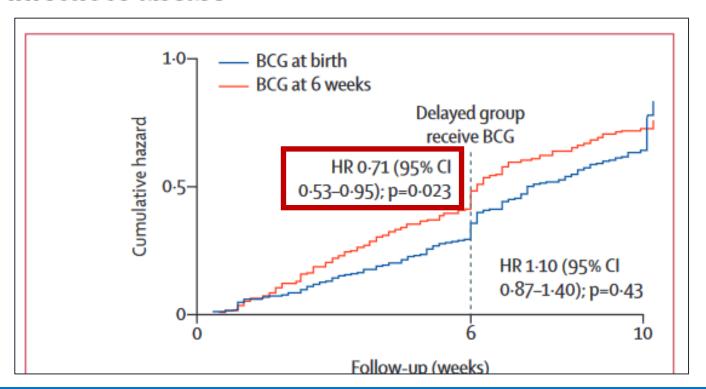
Randomised trials: BCG-Denmark at birth or the usual delayed BCG in LBW neonates: Effect on neonatal mortality



Combined analysis 38% (17-54%) reduction in all-cause neonatal mortality
Mainly due to *non-specific protection* against septicaemia and respiratory infections
Biological gradient with lower effect with lower all-cause mortality supports a biological effect

Randomised trial: BCG-Denmark at birth or delayed BCG: Effect on non-TB morbidity in Uganda

Figure 2: Cumulative hazard of physician-diagnosed, non-tuberculous infectious disease



BCG+OPV vs OPV: 29% (5-43%) reduction in non-TB infectious diseases up to 6 weeks of age (when control group gets BCG)

Cluster-randomised trial: BCG-Japan+OPV at birth or delayed: Effect on mortality in rural Guinea-Bissau

BMJ Global Health

Can earlier BCG-Japan and OPV vaccination reduce early infant mortality? A cluster-randomised trial in Guinea-Bissau

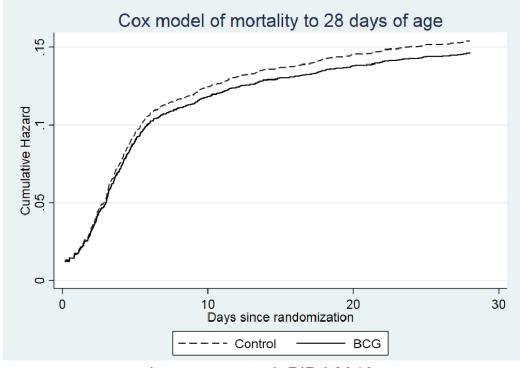
Fable 2 Effect of providing BCG and OPV at a home visit on non-accidental mortality and potential effect modifiers							
	n	MR/1000 PYRS (deaths/PYRS)	HR (95% CI)				
Main outcome: non-accidental mortality							
Intervention	1006	66.5 (7/105)	0.41 (0.18 to 0.92)				
Control	1206	276.5 (28/101)	Reference				

BCG+OPV vs no vaccines: 59% (8-82%) reduction in risk of non-accident mortality until next visit

RCT from India: BCG-Russia strain at birth or delayed: Effect on mortality

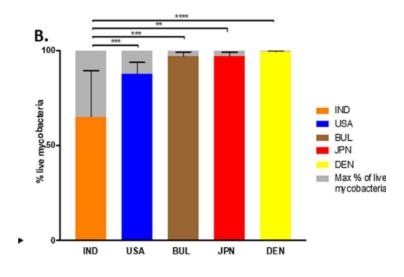
VACCINE REPORTS

Two Randomized Trials of the Effect of the Russian Strain of Bacillus Calmette-Guérin Alone or With Oral Polio Vaccine on Neonatal Mortality in Infants Weighing <2000 g in India



Jayaraman et al, PIDJ 2019

Licensed Bacille Calmette-Guérin (BCG) formulations differ markedly in bacterial viability, RNA content and innate immune activation

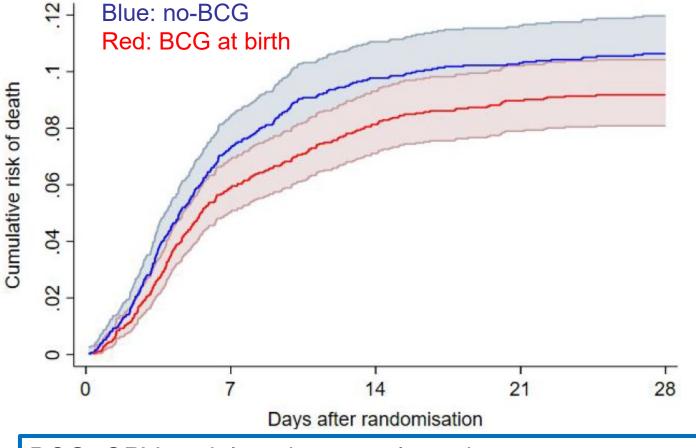


Angelidou et al, Vaccine 2020

No effect of BCG-Russia on neonatal mortality

But BCG-Russia, both for specific and non-specific effects, is a weak strain of BCG

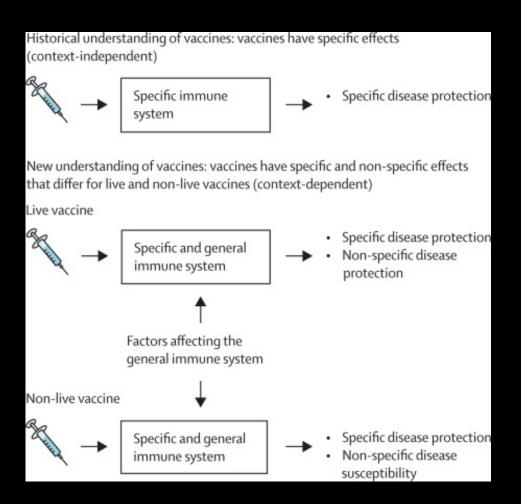
New RCT from India: BCG-Denmark+OPV at birth or delayed: Effect on mortality



Meta-analysis of all BCG-Denmark trials, Primary outcome: 19% (7-28%) reduction. Neonatal mortality: 23% (8-36%) reduction.

BCG+OPV vs delayed neonatal vaccines: 17% (2% to 31%) reduction in neonatal all-cause mortality 47% (30% to 60%) reduction in neonatal infectious disease mortality

Important insight: Vaccine effects are context-dependent



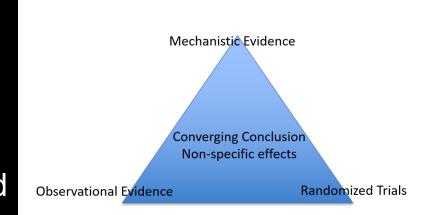
Effect modifiers of both specific and non-specific effects: Sex, age, vaccine strain

Additional effect modifiers of non-specific effects: maternal priming, boosting, other vaccines, vitamin A

Aaby, Netea & Benn, Lancet Infect Dis 2022

Triangulating data on BCG

- Repeated observational studies: BCG associated with ~50% reduction in all-cause child mortality
- Could be healthy vaccinee bias?
 - Meta-analysis: Among BCG vaccinated children: those w/ scar
 39% lower mortality than those wo/scar
 - Opposite effect of different vaccines
 - RCTs: 17-59% reduction in all-cause mortality
- Not consistent in all studies?
 - Effect modifiers consistent in explaining divergent results: vaccine strain, maternal priming
- Immunological mechanism: BCG induced trained innate immunity



Støvring enters the scene at Weekendavisen

Henrik Støvring, statistikprofessor på Aarhus Universitet, har samlet alle de videnskabelige artikler om studier med vacciner udført af Aaby og Stabell Benn, som han kunne finde.

Title

Evaluating the effect of BCG vaccination for non-specific protection fro Effect of a campaign with oral polio vaccine on general health: A cluste Effects of Neonatal BCG-Japan Versus BCG-Russia Vaccination on O Can earlier BCG-Japan and OPV vaccination reduce early infant morta Using BCG Vaccine to Enhance Nonspecific Protection of Health Care Effect of early two-dose measles vaccination on childhood mortality and The Effect of a Second Dose of Measles Vaccine at 18 Months of Age The mortality effects of disregarding the strategy to save doses of mea Early Vaccination With Bacille Calmette-Guérin-Denmark or BCG-Japa BCG Vaccination at Birth and Rate of Hospitalization for Infection Until Early BCG-Denmark and Neonatal Mortality Among Infants Weighing No effect of an additional early dose of measles vaccine on hospitalizat A Two-Center Randomized Trial of an Additional Early Dose of Measles Neonatal BCG vaccination and atopic dermatitis before 13 months of a Bacillus Calmette-Guérin vaccination at birth and in vitro cytokine response Effect of early measles vaccine on pneumococcal colonization: A rand Nonspecific effect of BCG vaccination at birth on early childhood infec BCG vaccination at birth and early childhood hospitalisation; a random Adverse reactions to the Bacillus Calmette-Guérin (BCG) vaccine in ne Early BCG vaccine to low-birth-weight infants and the effects on growth The Effect of Oral Polio Vaccine at Birth on Infant Mortality: A Random High-dose vitamin A with vaccination after 6 months of age: a randomiz Vitamin A supplementation and risk of atopy: long-term follow-up of a ra Vitamin A supplementation and BCG vaccination at birth may affect ato Small randomized trial among low-birth-weight children receiving bacillo Randomized trial of BCG vaccination at birth to low-birth-weight children Effect of revaccination with BCG in early childhood on mortality: rando Vitamin A supplementation and BCG vaccination at birth in low birthwei Effect of 50,000 IU vitamin A given with BCG vaccine on mortality in inf Non-specific effects of standard measles vaccine at 4.5 and 9 months

Found an effect on primary outcome

Effect on prespecified secondary outcome

Found no direct effect on primary and secondary outcomes

Interim analysis / trial stopped before time

Part of another trial on the list - not separate trial

Not about vaccines

Alle på nær én af de studier er nulfund. Det vil sige, at de afviser de hypoteser, de undersøger, herunder hypotesen om uspecifikke vaccineeffekter.

Støvring presented a list of 30 so-called "nulf-findings" that allegedly refuted the hypothesis of non-specific effects But among others:

- → multiple studies with effect on primary and secondary outcomes
- → multiple studies of vitamin A and multiple substudies of some RCTs

Found an effect on primary outcome

Effect on prespecified secondary outcome

Found no direct effect on primary and secondary outcomes

Interim analysis / trial stopped before time

Part of another trial on the list - not separate trial

Not about vaccines

Støvring et al's new paper



Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Commentary

What is actually the emerging evidence about non-specific vaccine effects in randomized trials from the Bandim Health Project?

Henrik Støvring ^{a,b,c,*,1}, Claus Thorn Ekstrøm ^{d,2}, Jesper Wiborg Schneider ^{e,3}, Charlotte Strøm ^{f,4}

mean power, possibly due to <u>questionable research practices</u> such as a lack of adjustment for multiple comparisons, selection bias, p-hacking, or subsequent publication bias.

Arbitrary selection of our papers

In contrast to the Weekendavis list:

- Glad to see vitamin A papers left out!
- Many of the substudies to the Calmette study left out

But:

- No studies before 2008 (e.g., not the high-titre measles vaccine trials)
- Arbitrary selection of substudies: some RCTs have >5, others none though they exist

Errors in extracting number of tests

Double counted estimates and p-values

Counted both estimate and reference estimate of 1.00 as test

		All (n = 467)				
		Obs in Range	GMR (95% CI)		P Value	
Medium	IL-1β	85%	1.33 (.97–1.83)		.08	
	IL-6	87%	1.27 (.90–1.80)		.17	
	TNF-α	96%	1.30 (1.05–1.60)		.01	
	IL-5	60%	0.98 (.77–1.25)		.86	
	IL-10	76%	1.13 (.85–1.52)		.40	
	IL-17	62%	1.00 (.73–1.38)		.98	
	IFN-γ	82%	1.40 (1.04–1.88)		.03	

Table 2 Mortality and HRs for death among children eligible for enrolment in the MVEPI trial. Overall and eligibility assessment									
	N	Deaths/person years (PYRS)	Mortality rate (pe	er HR 95% CI*					
Children 12–35 months at eligibility assessment									
Restrictive MV policy	1373	44/3698	11.9	1.00 (ref)					
MV for all	1405	45/3723	12.1	0.95 (0.64 to 1.43)					
All Children									
Restrictive MV policy	2339	81/6775	12.0	1.00 (ref)					
MV for all	2428	92/6983	13.2	1.06 (0.78 to 1.44)					

Multiple examples of wrongly counted tests. E.g.,:

- → the same analysis represented by four estimates from unadjusted/adjusted and intention-to-treat and *per protocol* analyses counted as four independent tests
- → tests comparing background characteristics
- in direct violation of the principles for Holm-Bonferroni correction: it controls family-wise error *only* if tests are independent (and relevant)

Errors in the interpretation of their own results

PaperID 4: Five estimates hold op for HB correction

per	ID Paper	origin	Publ used MC?	Estimate	e LCL	UCL	Reported p-value	HB_pval	Holm-B significant p value?
4 E	Biering-Sørensen, S., Aaby, P.,								No
J _{4 E}	Biering-Sørensen, S., Aaby, P.,	Table 2							Me
4 E	Biering-Sørensen, S., Aaby, P.,	Table 3			0.57	0.35	0.93		0.02 Yes
4 E	Biering-Sørensen, S., Aaby, P.,	Table 3			0.37	0.17	0.84		0.03 Yes
4 E	Biering-Sørensen, S., Aaby, P.,	Figure 3			0.55	0.34	0.89		0.13 No
4 E	Biering-Sørensen, S., Aaby, P.,	Figure 3						0.03	0.11 No
4 E	Biering-Sørensen, S., Aaby, P.,	Figure 3						0.03	0.10 No
4 E	Biering-Sørensen, S., Aaby, P.,	Figure 3						0.00	0.01 Yes
4 E	Biering-Sørensen, S., Aaby, P.,	Figure 3						0.00	0.01 Yes
4 E	Biering-Sørensen, S., Aaby, P.,	Figure 3						0.05	0.05 Yes
4 E	Biering-Sørensen, S., Aaby, P.,	Figure 3						0.04	0.07 No

Not paperID 4 – maybe paperID 5??

Yet, <u>in the very same table</u> it is concluded that the HB results do <u>not</u> support non-specific effects?

The same is the case for 7 other studies

Paper reports primary results for RCT	Results support primary finding of NSE (only relevant if N=Yes)	HB Results support primary finding of NSE (only relevant if N=No)		Authors primary conclusion is finding of a NSE	Authors secondary conclusion is finding of a NSE	Year of publication	Has trial ID	Analysis comparing randomization arms
Yes	No		No	Yes	Yes	2017	Yes	Yes

Flawed reading of our papers

Støvring et al claim only 1/13 RCTs found significant effect on <u>primary outcome</u> – but 4/13 RCTs included had significant effect on the primary outcome

Example:

Effect of early two-dose measles vaccination on childhood mortality and modification by maternal measles antibody in Guinea-Bissau, West Africa: A single-centre open-label randomised controlled trial

Sebastian Nielsen, ^{a,b} Ane B Fisker, ^{a,b} Isaquel da Silva, ^a Stine Byberg, ^a Sofie Biering-Sørensen, ^a Carlitos Balé, ^a Amarildo Barbosa, ^a

Background Early 2-dose measles vaccine (MV) at 4 and 9 months of age vs. the WHO strategy of MV at 9 months of age reduced all-cause child mortality in a previous trial. We aimed to test two hypotheses: 1) a 2-dose strategy reduces child mortality between 4 and 60 months of age by 30%; 2) receiving early MV at 4 months in the presence versus absence of maternal measles antibodies (MatAb) reduces child mortality by 35%.

OPV before and after enrolment (p for interaction=0.027) [deaths/children: $n_{2\text{-dose}}$ =27/1,602; $n_{1\text{-dose}}$ =3/837]. In the 2-dose group receiving early MV at 4 months, mortality was 50% (20–68%) lower for those vaccinated in the presence of MatAb vs. the absence of MatAb [deaths/children: n_{MatAb} =51/3,132; n_{noMatAb} =31/1,028].

Flawed reading of our papers

Støvring et al claim that we interpreted findings on <u>secondary outcomes</u> as causal in 23/25 papers – <u>but</u> we use cautious language Example:

BCG Vaccination at Birth and Rate of Hospitalization for Infection Until 15 Months of Age in Danish Children: A Randomized Clinical Multicenter Trial

Lone Graff Stensballe, Henrik Ravn, Nina Marie Birk, Jesper Kjærgaard, Thomas Nørrelykke Nissen, Gitte Thybo Pihl, Lisbeth Marianne Thøstesen,

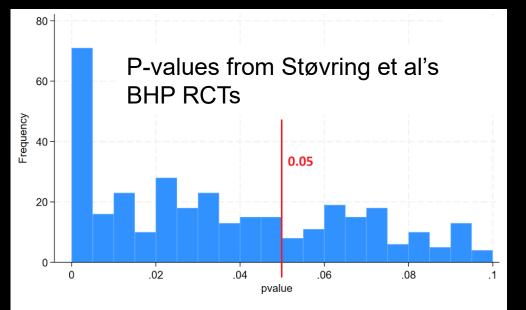
Conclusion. BCG vaccination did not affect the rate of hospitalization for infection up to the age of 15 months in Danish children. In future studies, the role of maternal BCG-vaccination, premature birth, and cesarean delivery needs further exploration.

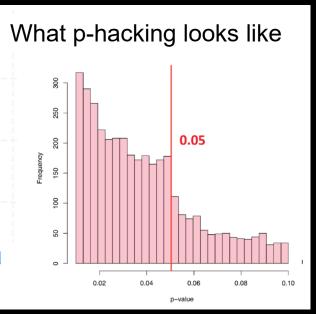
Violating assumptions for Z-curve methodology

Støvring et al. applied Z-curve to >1,400 statistical tests drawn from 26 publications representing only 13 unique RCTs (concluding that there are indications of p-hacking). This violates core methodological principles required for valid Z-curve inference:

- The Z-curve methodology requires many independent studies
- Z-curve assumes one p-value per study; Støvring et al. heavily overweight single RCTs by including hundreds of internally dependent tests within the same RCT

A simple way to analyse p-hacking was not applied - But would have contradicted p-hacking





Summary scientific assessment

Støvring et al's new paper

- Unusual in its lack of clear research question
- Error prone: hundreds of errors in extracting tests, flawed reading of their own results, flawed reading and interpretation of our papers, violated methodological assumptions.
- No consideration of the totality of data from us and others.
- Somewhat belated to claim that we have not shown non-specific effects as these effects undoubtedly exist, and hundreds of researchers worldwide study them and find supportive evidence.







Webinar

The latest news on the +100-year-old BCG-vaccine against tuberculosis

A brief update on new studies

Date: 8 January 2026 - from 12.30 pm - 2.30 pm CET

Link: https://syddanskuni.zoom.us/j/68200560376?pwd=Sh5hZq5uQjankfdooY18dHSvfRLtZ3.1

Brief introduction: Welcome to our webinar on the latest developments in the non-specific effects of the Bacillus Calmette–Guérin (BCG) vaccine. Traditionally known for its role in protecting against tuberculosis, the BCG vaccine has increasingly attracted attention for its broader immunological benefits. Emerging evidence suggests that BCG vaccination may enhance resistance to unrelated infections and even modulate immune responses to other diseases, including certain chronic diseases. In this webinar, we will explore the most recent findings from clinical and immunological studies. Join us for an engaging discussion at the intersection of epidemiology, immunology, and public health innovation.

Program:

- 12.30 pm CET: Dean of Health Ole Skøtt and Professor Christine Stabell Benn, University of Southern Denmark: Short welcome addresses
- 12.35-1.00 pm: Professor Bethou Adhisivam, JIPMER, India: Effect of BCG on neonatal mortality in a randomised trial in India and an overview of BCG's effect on neonatal mortality
- 1.00-1.25 pm: Professor Denise Faustman, Mass General Research Institute: Phase II RCT in diabetes with Multi-dose BCG: Outcomes of Alzheimer's Disease
- 1.25-1.45 pm: A/Prof Laure F Pittet, The University of Melbourne & University of Geneva: Neonatal BCG vaccination decreases eczema incidence at 5 years: the MIS BAIR randomised controlled trial and an overview of BCG's effect on atopic dermatitis
- **1.45-2.00 pm: Professor Steven Josefowicz, Weill Cornell Medicine:** BCG's effect on circulating haematopoetic stem cells in Danish children
- 2.00-2.10 pm: A/Professor Asimenia Angelidou, Harvard University: BCG vaccination at birth reprograms the neonatal plasma metabolome in vivo and in vitro: implications for early life trained immunity
- **2.10-2.25 pm: Dr. Frederik Schaltz-Buchholzer, University of Southern Denmark:** BCG's effect on all-cause mortality in adults: A meta-analysis of randomised trials during the pandemic

There will be 5 min set aside for Q/A for each presentation.



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hybrid conference

Health Effects of Vaccines: From Genomics to Policy

11–13 May 2026 | Hinxton Hall Conference Centre Wellcome Genome Campus, UK or online

Deadlines: Bursary - 2 February | Abstract - 2 March Registration - 13 April | Virtual registration - 4 May









The discussion has moved beyond the scientific arena with personal attacks and defamatory statements with obvious negative consequences for our work in Guinea-Bissau

Why? Who benefits?

Opløsning. Statistikprofessorer hiver med ny analyse tæppet væk under kendt vaccinepars mange lodtrækningsforsøg. De er oversolgte og støtter ikke hypotesen om uspecifikke vaccineeffekter, slår professorerne fast.



Den nye undersøgelse er den første, der systematisk analyserer på tværs af alle Christine Stabell Benn og Peter Aabys lodtrækningsforsøg. Hvor andre tidligere har kritiseret enkelte studier, har forskerne bag den nye gennemgang set på det samlede billede.

– Vi finder tegn på, at forskerne systematisk har plukket og fremhævet resultater, der passede deres teorier, mens de har nedtonet, at de ikke fik bekræftet den primære hypotese, som forsøgene egentlig skulle undersøge. Når man ser på det samlede billede, er der stort set ingen reelle fund tilbage, forklarer Henrik Støvring.

Scientific fruitful way forward

NOT:

- Refuting open scientific discussion on equal terms (our main tool as academics)
- Writing flawed paper with personal attacks
- Working with a journalist and issuing press statements based on same flawed paper

YES TO:

- Scrutiny
- Looking at the totality of data triangulation is a valuable tool
- Polite and curious scientific discussions of which hypotheses fit the totality of data
- "We encourage quantitative and statistical thinking, not mindless statistics" (Schneider 2013)

Thank you!



Mothers and children in Guinea-Bissau

Our local staff of ~170 assistants, drivers, nurses, doctors and lab technicians and TAPs



Bandim Health Project: Peter Aaby

Ane Fisker
Signe Sørup
Kristoffer Jensen
Sanne Thysen
Frederik Schaltz-Buchholzer
Andreas Rieckmann
Amabelia Rodrigues
Cesario Martin
Isaquel da Silva
Elsi Ca

Statisticians:

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