Sigurveig Þóra Sigurðardóttir, MD

Pneumococcal conjugate vaccines in Icelandic infants.

Safety, immunogenicity and protective capacities.

Supervisor:

Professor Ingileif Jónsdóttir, Fil.Dr.

PhD Thesis

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Supervisor: Professor Ingileif Jónsdóttir, Fil.Dr.

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ÁGRIP

Inngangur. Pneumókokkar (*Streptococcus pneumoniae*) eru bakteríur sem eru algeng orsök sjúkdóma og dauðsfalla meðal ung- og smábarna. Pneumókokkar eru hjúpaðir fjölsykrum (pneumococcal polysaccharides, PPS) sem vekja mótefnasvar óháð T frumum og valda því hvorki flokkaskiptum mótefna né ónæmisminni og eru ekki ónæmisvekjandi í ungbörnum. PPS tengdar próteini vekja hins vegar T-frumuháð mótefnasvar og eru ónæmisvekjandi hjá ungum börnum.

Markmið rannsóknanna sem þessi doktorsritgerð byggir á var að rannsaka ný bóluefni úr próteintengdum pneumókokkafjölsykrum (pneumococcal conjugate vaccine, PCV) í íslenskum ungbörnum og svara eftirfarandi spurningum: Eru PCV örugg og ónæmisvekjandi? Ræsa PCV myndun á virkum og verndandi mótefnum? Er hægt að hafa áhrif á mótefnasvarið með mismunandi burðarpróteinum? Er hægt að auka mótefnasvarið með því að tengja sömu fjölsykru við tvö prótein og blanda saman í eitt bóluefni? Er fullnægjandi að bólusetja ungbörn með tveimur skömmtum af PCV á fyrsta ári í stað þriggja? Vekur PCV ónæmisminni til skemmri og lengri tíma? Getur endurbólusetning með fullum skammti af 23-gildu fjölsykrubóluefni (PPV23) haft skaðleg áhrif á langtíma ónæmisminni? Hefur PCV áhrif á bólfestu pneumókokka í nefkoki, tíðni miðeyrnabólgu og notkun sýklalyfja?

Efniviður og aðferðir: Heilbrigð ungbörn voru bólusett með PCV á sama tíma og hefðbundin bóluefni voru gefin. Fjölsykra af hjúpgerð 6B tengd afeitruðu stífkrampa próteini (6B-TT) var gefin tveimur aldurshópum ungbarna og fullorðnum til samanburðar á mótefnasvari. Tvö burðarprótein voru borin saman í slembirannsókn á 8 hjúpgerðum sem voru tengdar annað hvort við afeitrað prótein barnaveikibakteríu (PCV8-DT) eða TT (PCV8-TT) og var endurbólusett með sama PCV eða fullum skammti af PPV23. PCV með 11 hjúpgerðum tengdum DT og/eða TT (F3bis: bæði DT og TT fyrir hjúpgerðir 6B, 9V, 18C og 23F) var rannsakað í börnum á öðru ári og í ungbörnum þar sem F3bis var borið saman við 11-gilt F3, þar sem hver hjúpgerð var bundin annað hvort DT eða TT. Borið var saman að bólusetja með tveimur eða þremur skömmtum af PCV með 9 hjúpgerðum pneumókokka og hjúpsykru meningókokka af gerð C, tengdum afeitruðu stífkrampapróteini “cross reacting material 197” (9vPnCMnCC) og endurbólusetningu með sama bóluefni eða PPV23. Samanburður á milli bóluefna eða fjölda skammta var gerður með slembun. Öryggi var metið með dagbókarfærslum. Mótefni voru mæld með ELISA fyrir og eftir frum- og endurbólusetningu og virkni mótefna metin *in vitro* með átfrumuprófi og *in vivo* með mati á vernd gegn pneumókokkasýkingum í músum. Langtímaminni gegn PCV-TT og áhrif endurbólusetningar með fullum skammti af PPV23 við 13 mánaða aldur á ónæmisminni var rannsakað við 7 ára aldur. Nefkoksræktanir voru teknar á aldrinum fjögurra til 30 mánaða. Sýklalyfjanotkun og tíðni miðeyrnabólgu hjá börnum undir tveggja ára var borin saman hjá bólusettum og óbólusettum börnum.

Niðurstöður: Öll bóluefnin voru örugg. Ónæmisvekjandi eiginleikar 6B-TT sáust í mótefnamyndun og minnissvari í tveimur aldurshópum bólusettum frá þriggja eða sjö mánaða aldri. Mótefnasvar af IgG1 gerð var ráðandi hjá ungbörnunum og eftir endurbólusetningu við 18 mánaða aldur höfðu þau sambærilegt magn af IgG1 og fullorðnir og IgG2 var einnig mælanlegt. Mótefnin stuðluðu að upptöku átfrumna á

pneumókokkum og drápi *in vitro*. Áttgildu bóluefnin PCV-DT og PCV-TT vöktu myndun sértækra IgG mótefna sem voru virk *in vitro* og vernduðu mýs gegn blóðsýkingu og lungnabólgu af völdum pneumókokka af hjúpgerð 6B. Ellefugildu bóluefnin F3bis og F3 vöktu marktæka myndun virkra IgG mótefna. Notkun tveggja burðarpróteina fyrir lítt ónæmisvekjandi fjölsykrurnar fjórar jók ekki mótefnasvarið. Bæði tvær og þrjár frumbólusetningar með PCV vöktu marktæka myndun IgG gegn öllum 9 hjúpgerðunum í 9vPnCMnCC bóluefninu. Þrír skammtar ollu hærra IgG frumsvari gegn sjö hjúpgerðum af níu en minnissvar við 12 mánaða aldur var svipað fyrir allar hjúpgerðir.

Sýnt var fram á ónæmisminni gegn PCV8-DT og PCV8-TT með marktæku IgG svari fjórum vikum eftir PPV23 endurbólusetningu við 13 mánaða aldur og gegn 9vPnCMnCC sem olli háu IgG endursvari strax viku eftir PPV23 við 12 mánaða aldur. Sýnt var fram á langtímaminni við sjö ára aldur hjá börnum sem höfðu verið bólusett með PCV8-TT sem ungbörn. Viðvarandi minni var til staðar hvort sem börnin höfðu fengið PCV eða PPV23 endurbólusetningu við 13 mánaða aldur sem ekki sást hjá börnum sem aldrei höfðu fengið PCV. PPV23 endurbólusetning við 13 mánaða aldur hindraði því ekki minnissvar við 7 ára aldur, en hlutfall IgG1/IgG2 var lægra.

Nefkoksræktanir sýndu lækkun í beratíðni pneumókokka af þeim hjúpgerðum sem voru í bóluefnunum en áhrifin voru horfin við 2 ára aldur. PCV bólusetning dró úr tíðni eyrnabólgu og notkun sýklalyfja hjá 18-24 mánaða gömlum börnum. Börn sem báru endurtekið hjúpgerðir 6B eða 23F í nefkoki reyndust hafa marktækt lægra magn af IgG í sermi gegn þessum hjúpgerðum en þau börn sem aldrei ræktuðust með sömu hjúpgerðir.

Ályktun. Sex próteintengd fjölsykrubóluefni gegn pneumókokkum reyndust örugg og vöktu myndun sértækra mótefna hjá íslenskum börnum, sem voru virk gegn pneumókokkum *in vitro* og *in vivo*. Þótt frumbólusetning með tveimur skömmtum vekti lægra mótefnasvar en þrír skammtar þá gaf hún marktækt mótefnasvar og minnismyndun. Endurbólusetningar á öðru aldursári og sex árum síðar sýndu viðvarandi ónæmisminni sem eyddist ekki af fullum skammti af PPV23 á öðru aldursári. Niðurstöðurnar benda til þess að bóluefnin geti varið ungbörn gegn alvarlegum sýkingum af völdum pneumókokka. Með almennri notkun próteintengdra fjölsykrubóluefna gegn pneumókokkum hjá ungbörnum geta þessi bóluefni komið í veg fyrir mikinn fjölda dauðsfalla og slæma fylgikvilla pneumókokkasýkinga.

Lykilorð: próteintengd fjölsykrubóluefni gegn pneumókokkum, ungbörn, öryggi, ónæmisvekjandi eiginleikar, ónæmisminni

ABSTRACT

Background: *Streptococcus pneumoniae* (pneumococcus) is a major pathogen causing infection and death among infants and young children. Pneumococcal capsular polysaccharides (PPS) are T-cell independent antigens, unable to induce isotype switching and immunological memory in infants whereas PPS conjugated to proteins are T-cell dependent antigens, immunogenic at an early age.

The aim of the studies behind this PhD thesis was to investigate in Icelandic infants new pneumococcal conjugate vaccines (PCVs) asking the following questions: Are PCVs safe in infants? Do PCVs induce functional antibodies in infants? Can antibody responses to PCV be influenced by different protein carriers? Can immunogenicity of PCV be enhanced by mixing two conjugates of the same PPS with two different protein carriers? Can infants be successfully vaccinated with two primary PCV doses compared to three? Does PCV induce short-term and long-term immunological memory and does a booster with a 23-valent pneumococcal polysaccharide vaccine (PPV23) compromise the long term immunological memory? Does the PCV vaccination affect the nasopharyngeal carriage of pneumococci or influence the rate of otitis media and use of antibiotics?

Methods: Healthy infants were vaccinated with PCV administered concomitantly with regular infant vaccines. Serotype 6B-tetanus toxoid conjugate (6B-TT) was given to two infant age groups of different ages and compared to adults. Two protein carriers were compared by randomizing 8-valent diphtheria toxoid (PCV8-DT) or tetanus toxoid (PCV8-TT) PCVs and a booster with the same PCV or a full dose PPV23. An 11-valent DT and/or TT PCV (F3bis; two carriers for 6B, 9V, 18C and 23F) was first investigated in toddlers and then compared in infants with an 11-valent F3 single DT/TT carrier PCV. Two vs. three infant PCV doses were compared in a study on a 9-valent pneumococcal/meningococcal C saccharides conjugated with the cross reacting material 197 (CRM_{197}) (9vPnCCMnCC) and a booster with PCV or PPV23. The comparison between vaccine formulations was done in a randomized design. The side-effects were recorded. Antibodies were measured by ELISA before and after primary and booster vaccinations and protective capacities evaluated *in-vitro* by opsonophagocytosis and *in vivo* in a mouse model of pneumococcal infection. Long-term memory to PCV-TT and the effect of full dose PPV23 booster at 13 months on immunological memory was investigated at 7 years of age. Nasopharyngeal cultures were obtained between 4 – 30 months of age. History of acute otitis media (AOM) and antibiotic treatments were compared with unvaccinated controls at 2 years of age.

Results: All the vaccines were safe. Immunogenicity of 6B-TT was demonstrated by antibody production and memory type of response in infants starting from 3 or 7 months of age. Antibody responses were dominated by IgG1 in infancy, and after booster at 18 months of age, the infants had adult levels of IgG1 and IgG2 started to appear. The antibodies were functional *in vitro* as shown by opsonophagocytosis. The 8-valent PCV-DT and PCV-TT induced serotype specific IgG that were functional *in vitro* and protective against serotype 6B bacteremia and lung infection *in vivo* in the mouse model. The 11-valent F3 and F3bis formulations induced significant functional IgG antibodies.

No benefit was observed from mixed double carrier formulation for the poorly immunogenic serotypes.

Both two and three primary PCV doses induced significant IgG to all pneumococcal serotypes in the 9vPnCMnCC. Three doses induced higher primary IgG to seven out of nine serotypes but memory responses at 12 months were similar.

Immunological memory was demonstrated to PCV8-DT and PCV8-TT by significant IgG responses four weeks after a PPV23 booster at 13 months and to 9vPncCMnCC with a brisk IgG response as soon as one week after the PPV23 booster at 12 months. Long-term memory was demonstrated in 7-year-old children primed with PCV8-TT in infancy who responded to a 1/10 PPV23 dose with a significant rise in IgG in one week. A persistent memory was demonstrated, whether they had received PCV8-TT or PPV23 booster at 13 months of age, which was not observed in PCV naïve children. The PPV23 booster at 13 months did not significantly compromise the memory response at 7 years of age but a lower IgG1/IgG2 was observed.

An initial decrease in nasopharyngeal colonization with vaccine serotypes was observed but that effect waned by the age of 2 years when carriage was comparable between PCV vaccinated children and unvaccinated controls. Medical history at 2 years showed fewer AOM and antibiotic treatments between 18 and 24 months compared to controls. Children, repeatedly colonized after vaccination had significantly lower IgG antibodies to the colonizing serotype compared to children never colonized with same serotype.

Conclusion. The six pneumococcal conjugate vaccines tested were shown to be safe when used in Icelandic children. They induced functional antibody responses when administered concomitantly with regular infant vaccinations. Although primary vaccinations with two doses resulted in lower antibody levels than three doses, it induced significant antibody responses and immunological memory. Booster vaccinations during second year of life and six years later demonstrated sustained immunological memory that was not exhausted by a full dose of PPV23 during the second year of life. The results support the protective potential of these vaccines against invasive pneumococcal diseases in the young. With general usage of protein conjugated pneumococcal vaccines in infants, these vaccines can prevent numerous deaths and other serious consequences from pneumococcal infections.

Keywords: pneumococcal-conjugate-vaccine (PCV), infants, safety, immunogenicity, memory.

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DECLARATION OF CONTRIBUTION

Together with Ingileif Jonsdottir, I was the principal investigator on all the clinical trials on which this thesis is based. As such I participated in the development of the research questions, study design, analysis of the results and preparation of data for presentations and publications.

I was responsible for all communication with the Icelandic authorities including the appropriate ethics committee for each study, the Data Protection Authority and the Icelandic Medicines Control Agency. I led the organization of the clinical part of the studies, found collaborators within the Primary Health Care System in Reykjavik, Kopavogur and Hafnarfjordur as well as within the Children's Hospital and Obstetrical ward at the Landspitali University Hospital. I was in charge of recruiting the staff, organizing and running the clinical work at the centres but in close collaboration with the collaborators also performing clinical work in different studies; Katrin Davidsdottir at the Centre for Child Health Services in study II - V, Vilhjalmur Ari Arason in Hafnarfjordur and Olof Jonsdottir in Kopavogur in study V, Thorolfur Gudnason and the local staff at Arbaer Health Centre, Reykjavik, Sveinn Kjartansson at Midbaejarstod, Reykjavik and the local staff at Solvangur in Hafnarfjordur in study II. I worked as a study physician in all the trials by seeing participating infants, performing well child care examination and immunizations besides administering the trial vaccines and obtaining blood samples and nasopharyngeal swabs for culturing at all the study centres. Ingileif Jonsdottir was in charge of all laboratory work performed at the Dept. of Immunolgy, Landspitalinn, including all serum antibody measurements in trials I - IV, sample preparations in trial V, functional assays and mouse protection model. Papers I and II are also part of Gestur Vidarsson´s PhD Thesis, University of Utrecht, The Netherlands. Steinn Jonsson was the PI of the collateral adult study investigating the same vaccine in collaboration with Ingileif Jonsdottir presented in papers I and II. In paper 3, my contribution was the conduction of the trial from which the sera were obtained, but Eirikur Sæland worked on the development of the mouse model and the paper is also part of his PhD Thesis from University of Utrecht, The Netherlands. Antibody measurements for trial V were done by Wyeth. Karl G. Kristinsson at the Department of Microbiology was responsible for the pneumococcal culturing and serotyping. Statistical analyses to answer primary and secondary objectives in each study were performed by the vaccine producers and Ingileif Jonsdottir was responsible for the analyses of the functional data and results on antibody characteristics. Statistics in the additional studies were performed by me including evaluation of nasophayngeal colonization, relationship between colonization and antibody responses and kinetics after booster in trial V. I have submitted abstracts, prepared and presented 10 posters and two oral presentations at international meetings. I have processed the data presented in this thesis and written or participated in the writing of the papers, on which this thesis is based.

ABBREVIATIONS

µg/mL:	microgram/millilitre
23vPPS:	23-valent-pneumococcal-polysaccharide-vaccine
6BTT:	Monovalent serotype 6B conjugated with tetanus toxoid
9vPnCMnCC:	9-valent pneumococcal conjugate (serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F) mixed with a meningococcus C conjugate, both conjugated with the CRM197 protein.
ANOVA:	Analysis of variance
AOM:	Acute otitis media
aP:	Acellular pertussis
CD4:	Surface glycoproteins on T helper cells
CI:	Confidence interval
CPS:	Cell wall polysaccharide
CRM197:	Cross reactive material 197, a mutant diphtheria toxoid
DT:	Diphtheria toxoid
DTaP:	Diphtheria, Tetanus and acellular Pertussis
DTwP/PRP-T:	Diphtheria, Tetanus, whole cell Pertussis / polyribosyl ribitol phosphate (Hib) conjugated with tetanus toxoid.
DTwP:	Diphtheria, Tetanus and whole cell Pertussis
ELISA:	enzyme-linked immunosorbent assay
FinOM:	Finnish otitis media efficacy trial.
GMC:	Geometric mean concentration
HbOC:	*Haemophilus influenzae* - CRM197 conjugate vaccine
Hi:	*Haemophilus influenzae*
Hib:	*Haemophilus influenzae* type b
HIV:	Human immunodeficiency virus
Ig:	Immunoglobulin
IgA:	Immunoglobulin A
IgG:	Immunoglobulin G
IgM:	Immunoglobulin M
IPV:	Inactivated polio vaccine
KPNC:	Keiser Permanente Northern California
MCPS:	Meningocccus C polysaccharide

MenA:	Meningococcus A
MenC:	Meningococcus C
MHC:	Major histocompatability complex
MMR:	Measles, mumps and rubella vaccine
MnCC:	Meningococcal C conjugate vaccine
NICHD:	National Institute of Child Health and Development
NP:	Nasopharyngeal
NS:	Not significant
NVT:	Nonvaccine serotypes
OM:	Otitis media
OMPC:	Outer membrante protein complex
OPA:	Opsonophagocytic Activity
PBS:	Phosphat buffered saline
PCV:	Pneumococcal conjugate vaccine
PCV11-F3-alum:	PVC formulation containing pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F, conjugated with diphtheria toxoid or tetanus protein with aluminum hydroxide adjuvant
PCV11-F3:	PVC formulation containing pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F, conjugated with diphtheria toxoid or tetanus protein.
PCV11-F3bis:	PVC formulation containing pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F, conjugated with diphtheria toxoid or tetanus protein. Serotypes 6B, 9V, 18C and 23F conjugated with both proteins.
PCV7-CRM197:	PVC formulation containing pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, conjugated with cross reactive material 197, a mutant diphtheria toxoid.
PCV8-DT:	PVC formulation containing pneumococcal serotypes 3, 4, 6B, 9V, 14, 18C, 19F, and 23F, conjugated with diphtheria toxoid
PCV8-TT:	PVC formulation containing pneumococcal serotypes 3, 4, 6B, 9V, 14, 18C, 19F, and 23F, conjugated with tetanus toxoid
PncD:	Serotypes 3, 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to diphtheria toxoid.
PncT:	Serotypes 3, 4, 6B, 9V, 14, 18C, 19F and 23F conjugated or tetanus protein.
PPS:	Pneumococcal polysaccharide vaccine
PRP-T:	Polyribosyl ribitol phosphate (*Haemophilus influenzae* antigen)

PS:	Polysaccharide
PsaA:	Pneumococcal surface adhesin A
PspA:	Pneumococcal surface protein A
RIA:	Radioimmunoassay
SAE:	Severe adverse event
T cell:	Thymus derived lymphocyte
TT:	Tetanus toxoid
VT:	Vaccine serotypes
VTP:	Vaccine type pneumococci
VRT:	Vaccine related serotypes
wP:	Whole cell pertussis

INTRODUCTION

General introduction

Streptococcus pneumoniae (pneumococcus) remains one of major pathogens causing infection and death among infants, young children and elderly. This is partly explained by the poorly immunogenic pneumococcal polysaccharide (PPS) encapsulating the pneumococcus. PPS are T-cell independent antigens that are poorly immunogenic and unable to induce isotype switching and immunological memory in young infants and elderly. In the development of vaccines against pneumococcus the PPS has been rendered immunogenic by conjugation to proteins, changing them to T-cell dependent antigens. Several formulations of pneumococcal protein conjugate vaccines (PCVs) have been designed and tested in clinical trials. The seven valent Prevnar®/Prevenar® has been until January 2009 the only registered PCV and is now widely used for infant vaccination.

This PhD is based on research on six formulations of PCVs with different number of serotypes, investigating their safety, immunogenicity and functional capacity of vaccine induced antibodies in Icelandic infants, comparing different carrier proteins and, persistence of immunological memory in toddlers and 6 years after primary vaccination of infants, as well as the effect on nasopharyngeal carriage and otitis media.

Streptococcus pneumoniae

Historical landmarks.

Streptococcus pneumoniae (pneumococcus) is a Gram positive bacterium, first recognized in infected sputum by Edwin Klebs in 1875 (reviewed in (1)). It was isolated for the first time 1880 by two scientists, George M. Sternberg and Louis Pasteur when they independently injected animals with saliva leading to deadly infection. Sternberg described the micrococci, joined in pairs and having diameter of 0,5µ. Pasteur described

the organism he saw under the microscope as having a certain aureole which he suggested that corresponded perhaps to material substance. This was later found to be the capsule that is central to pathogenesis and immunity of the pneumococcus (2, 3). The name "pneumokokkus" was given by Albert Fraenkel in 1887, same year as Anton Weichselbaum suggested the name *Diplococcus pneumoniae*. *Diplococcus pneumoniae* was used until 1974 when it was reclassified as *Streptococcus pneumoniae* based on its growth in chains in liquid media. With the help of Gram's discovery that gentian violet was retained in pneumococci after precipitation with iodine and washing with alcohol, Weichselbaum found in 1881 – 1886, pneumococcus in 94 out of 129 patients with pneumonia and pneumococcus was gradually accepted as the most common and most consistent pathogen causing pneumonia (reviewed in (1, 4)). Before the antibiotic era in early 20th century, pneumococcus was the third deadly cause, following heart diseases and cancer (1). The importance of the spleen in the resistance to pneumococcal infection was observed in the middle of the 20th century when several cases of severe invasive pneumococcal disease (IPD) following splenectomy were described and similarly children with sickle cell disease were later found to be at increased risk for IPD. The important role of gammaglobulins in pneumococcal defence was observed in 1952 when X-linked agammaglobulinemia was described by Bruton in a boy with severe IPD (reviewed in (1)). Avery, Heidelberger and Goebel discovered in 1920s the essential link between pneumococcal capsules and their serotype specificity.

The observations of Avery, Heidelbereger and Goebel led to the discovery of serotype specific antibody responses and vaccine development, containing serotype specific capsular polysaccharides. To date, 91 distinct capsular serotypes have been discovered (reviewed in (5, 6)). Each capsular polysaccharide has its unique serological property but several serotypes are cross-reactive, thus forming 46 serogroups. The polysaccharides are very long polymers of either linear or branched repeating units, consisting of two to eight monosaccharides. The antigenic epitopes of some of the PPS have been mapped which allows for identification of unique and cross-reactive sites within each serotype (6, 7).

The virulence factors.

The polysaccharide capsule of the pneumococcus is a major virulence factor. Besides shielding the inner structures of pneumococcus from the immune system (8), it enables the pneumococci to evade opsonization by the alternate complement pathway in the pre antibody phase of infection (9) by degradation and inactivation of C3 (10). The deposition and degradation of opsonic complement factors (C3b, iC3b, C3d) varies between serotypes which may partly explain variable immunogenicity between serotypes (11). Many pneumococcal proteins (pneumolysin, pneumococcal histidine triad proteins (Pht), factor H inhibitor of complement (Hic), pneumococcal surface protein C (PspC) also called cholin binding protein A (CbpA), pneumococcal surface protein A (PspA),in the cell wall are also known to interfere with complement-mediated opsonization and phagocytic killing of the bacteria (reviewed in (12)). Pneumococcal infection is characterized by a profound inflammation which by itself can lead to tissue damage. The cell wall components play a major role in generating the inflammation by activating the alternative complement system, inducing platelet activating factor and secretion of cytokines (13). Pneumolysin is a potent thiol-activated cytotoxin that produces pore formations in any cell membrane, leading to cell death (14). The immune response to the pneumolysin can be a double edged sword, inducing protective immune response but at same time inducing harmful pathological tissue changes.(15).

Epidemiology

The pneumococcus as a cause of morbidity and mortality in infants.

Despite the development and introduction of antibiotics, the pneumococcus remains a major cause of invasive and mucosal bacterial infections in infants and children. It causes significant infant mortality in the developing world (16-18) and is recognized as one of main causes of invasive bacterial infections in the western societies as well (19-21) with high case fatality rates (CFR) and severe invalidating sequelae. The WHO estimates the world wide annual rate of pneumonia in children less than 5 years of age to be around 150 million cases of which 716.000 die (22, 23). The total annual death due

to pneumonia is estimated to be around two million cases (22). Studies using lung aspirate for diagnosing etiology of pneumonia in children have found 10 – 77% aspirates to be positive to pneumococcus (24).

Invasive Pneumococcal Disease (IPD).

In table I, the incidence of IPD in infants in several European counties is presented. The potential life threatening invasive pneumococcal disease can present in the following

Table 1. European data on IPD in children under 2 years of age, collected prospectively in given country or area.

Country	Time period	Incidence of IPD and 95% CI (cases pr. 100.000/ year)	Reference
Denmark	1981 – 99.	34,91 (32.50 – 37.32)	Kaltoft et al. (26)
Danmark	2000 - 05	59.9 (CI not provided)	Harboe et al. (27)
Finland	1985 - 89	45.30 (39.99 – 50.60)	Eskola et al. (28)
Norway	1995 - 01	18.60 (16.21 – 20.98)	Pedersen et al.(29)
Norway - Oslo	1998 - 04	50 (n=14531 /6 years)	Brauteset et al. (30)
Southern Sweden	1981 - 95	28.7 (21.83 – 35.57)	Dahl et al. (31)
Sweden - Gothenburg	1981 - 96	9.4 (CI not provided)	Ekdahl et al. (32)
Spain - Valencia	1996 – 98	16.87 (12.53 – 21.21)	Diez-Domingo et al. (33)
Spain - Catalonia	1997 - 99	59.59 (51.10 – 68.09)	Domingues et al. (34)
Scotland	1988 – 99	13.86 (12.2 – 15.71)	Kyaw et al.(35)
Scotland	1999 – 01	31.73 (25.63 – 37.83)	Kyaw et al.(36)
Austria	2001 – 03	14.53 (10.28 – 18.77)	Rendi-Wagner et al. (37)
Germany	1997 – 98	23.11 (21.45 – 24.77)	Von Kries et al. (38)
UK - Nottingham	1980 – 99	37.80 (31.94 – 43.65)	Ispahani et al.(39)
UK - England and Wales	1996 – 98 < 1 year of age	39.7 (CI not provided)	Miller et al. (40)
Belgium	2002 – 03	104.4 (91.2 – 117.6)	Vergison et al. (41)
Germany	1997	8.1 (95% CI: 6.8; 9.7)	Rückinger et al. (42)
	2003	11.3 (95% CI: 9.7; 13.2)	

The table is partly adapted from (43) with additional data.

clinical forms; the bacteremic pneumococcal pneumonia, pneumococcal meningitis, bacteremia without a focus, septic shock, peritonitis, periorbital cellulitis and septic shock (25). Severe short- and long-term sequelae are common among survivors including deafness, nerve palsy, brain infarction, brain abscess, epileptic seizures and hydrocephalus (44-48). Less than half of patients recovering from pneumococcal meningitis recover fully (41, 46).

In USA the PCV vaccination was implemented in 2000 with infant vaccination. Before the PCV was introduced in general vaccination programs, data from community-based studies indicated that the overall annual incidence of pneumococcal bacteremia in children less than 2 years of age was 160 cases per 100,000. In adults, 60%-87% of pneumococcal bacteremia was associated with pneumonia but in young children, the primary sites of infection was frequently not identified (23). According to the surveillance systems in Europe the incidence of IPD is lower in Europe than USA (43, 49). The incidence of IPD is highest in the youngest age group and rises again in the elderly (27, 31, 32, 50). European data on IPD in children less than 2 years of age are summarized in Table 1. Prospective European studies before implementation of PCV have shown the incidence rates of IPD in children less than 2 years of age between 8.1 – 104 / 100.000 (26-40). A recent review of IPD and pneumococcal meningitis in Denmark showed an increase in the incidence of IPD in children less than 2 years of age between 2000 and 2005 (38.4 and 54.7/100.000, respectively (27). A similar trend was observed in other age groups. An older study from Sweden showed similar increase in IPD despite relatively constant numbers of blood cultures, from 5.2 / 100.000 in 1981 to 15.2 /100.000 in 1996 for all ages (32).

In USA an active core surveillance system has been in effect since 1995 (51). In Europe most countries have relied on voluntary passive reporting from microbiology laboratories. Surveillance for IPD in Europe is heterogeneous and large differences in reported disease incidence may reflect both true differences, and variations in patient and healthcare factors, including surveillance (52).The differences between active and passive surveillance systems may partly explain the difference between incidence data from USA and Europe (41). Other possible explanations may lay in different serotype

distribution (49), different threshold for blood culturing (53-55) or possibly differences in sizes of high risk groups and populations.

Pneumonia.

Lower respiratory tract infection (LRTI) is estimated to cause around 2 million deaths amongst children under 5 years of age (56). The majority of the deaths are in the developing countries (56) but LRTI also remain a major cause of childhood mortality in the industrialized countries (22, 56, 57). It is estimated that half of the deadly LRTI are caused by pneumococci (reviewed in (24)).

Acute otitis media (AOM).

AOM is one of the most common infections in childhood. It accounts for over 20 millions of paediatric medical visits each year in the USA (58) and is a major health problem in Europe as well as in the developing countries (59, 60). Approximately 60% of children experience one or more episodes of AOM during the first year of life (61-64). The pneumococcus along with nontypeable *Haemophilus influenzae* is the main bacterial cause of AOM (65, 66) and sinusitis (67). Infection at mucosal level is a risk factor for meningitis with 30% of pneumococcal meningitis being preceded by an ear infection, 20% by lung infection and 8% by sinusitis (68).

Nasopharyngeal colonization.

The pneumococcus colonizes normally the upper respiratory tract of almost every healthy individual at some time point in life. The nasopharynx being a normal reservoir for the pneumococcus is the port of entry for invasive disease. Asymptomatic carriage varies between populations, geographical areas and age, being the highest in toddlers, ranging from 43% - 88.7% (26, 50, 69-71). In the developing countries infants may become colonized soon after birth (72-77) while colonization of children in the developed countries is delayed by several months (69-71, 78-85). The serotype distribution varies also by age. The most common serogroups colonizing the nasopharynx belong to so called paediatric serogroups 6, 19 and 23 which are more common in children than in adults. They are poorly immunogenic and cause invasive pneumococcal disease in children (70, 75, 76, 79, 82, 86, 87). Pneumococci spread

easily between individuals in close environments such as within families and in day-care centers (71, 87-91). The duration of colonization varies widely and children can be colonized repeatedly with the same or different serotype and with more than one serotype at the same time (71, 73, 92). Because of the possibility of multiple colonizing serotypes and necessity for comparability between vaccine studies, a WHO consensus was reached on laboratory methods for detecting pneumococci in the nasophaynx (16, 93).

The mechanism of mucosal colonization by pneumococci is not fully understood but the fact that colonization precedes pneumococcal disease and that the nasophayngeal carriage serves as a reservoir for horizontal spread (78, 94) underscores the importance of this step in disease development.

The most common serotypes colonizing the nasophaynx of Icelandic children between 1992 and 1999 include serotypes 6A, 6B, 23F, 19F and 19A (87). There was a fluctuation of the three most prevalent colonizing serotypes, 6A, 6B and 23F (87) in children not vaccinated with pneumococcal conjugate vaccine (PCV). Similar data from Norway showed the same top four serotypes, 6A, 6B, 19F and 23F (50).

Pneumococcal serotypes causing infections.

Of the 91 recognized pneumococcal serotypes relatively few are responsible for the IPD in the world (20). Only three serogroups (6, 14 and 19) are responsible for over 50% of IPD in children (49). The pneumococcal serogroups contributing to majority of IPD in children in USA, Canada and Europe include 14, 6, 19, 18 and 23. Serotypes 1 and 5 are of importance in Africa and Asia. Studies on pneumococci colonizing nasopharyngeal mucosa in young children almost invariably rank serogroups 6, 14, 19 and 23 among the most prevalent ones, reviewed in (95).

In Iceland, all invasive bacterial pathogens are collected at the Department of Microbiology, Landspitali University Hospital in Reykjavik. In a retrospective study on IPD in Icelandic children, 0 – 18 years of age, from 1994 – 2005, 1253 positive blood cultures were reviewed. *S. pneumoniae* was cultured in 103 cultures from 97 children. The most prevalent serogroups/types were 14 (20%), 19 (19%) and 7 (12%) followed by 6B (9%), 23 (9%) 6 (6%), 6A (4%), 33 (3%), 18 (3%) and in 1-2% of cases 9, 4, 5, 16,

12 and 1 (96). Another Icelandic study looked at recurrent IPD in Iceland from 1975 to 2004 identifying only 12 children at median age of 2.5 years. In that study serotypes cultured were 6B, 7F, 9V, 14, 16, 18C, 23F, 19F, 19A and 33F (97).

An increasing prevalence of antibiotic resistant pneumococci has been of global concern for the last two decades. According to the Active Bacterial Core Surveillance program of the Centers for Disease Control and Prevention in the USA the proportion of IPD caused by penicillin resistant pneumococci increased from 21% in 1995 to 24% in 1998 (98). Infections due to multiresistent isolates increased also from 9% to 14% during the same period. Serotypes accounting for the majority of penicillin resistant pneumococci isolates were 19A, 9V, 6A, 23F, 6B, 19F and 14 (98). Prevalence of penicillin resistant pneumococci in Europe varies greatly between countries. High prevalence has been reported in Spain (45%) and France (25%), but lower in the UK (3%), Germany (8%) and Italy (5%) (99). In Iceland, the incidence of IPD caused by penicillin resistant pneumococci increased from 0% in 1988 to 19.8% in 1993, mainly in children less than 3 years of age (100). The penicillin resistant serogroups were mainly of 6, 19, and 23 (98.8%). Fifty two percent of nasopharyngeal cultures obtained from children attending five day care centers in Reykjavik between 1992 and 1999 were positive for pneumococci out of which 13.9% , mainly serotypes 19A and 6B, had decreased susceptibility to penicillin (87).

Immune responses

Mucosal immune responses

The natural infection by pneumococcus originates in the nasopharynx. It starts with asymptomatic carriage which in some cases leads to invasion and clinical disease. The balance between local defence mechanisms (84, 101, 102), competition with other organisms (103) and the invasiveness of the serotype (104, 105) determines whether the organism will successfully invade the body. Secretory IgA and locally leaked IgG against pneumococcal polysaccharides and surface associated proteins have been found in children who are colonized with pneumococcus (106, 107). The mucosal protection

however, involves other immune mechanisms under natural exposure as demonstrated in a mouse model showing the role of CD4+ T-cells (108, 109) and IL17 (110). Factors enhancing the elimination of the pneumococcus from the nasopharyngeal mucosa involve local innate immune responses in the nasopharynx which also plays a role in initiating adaptive immune responses (102). The recruitment of neutrophils and functional pneumococcal pneumolysin enhance antigen uptake in the nasal associated lymph nodes (84, 101, 111) which is an important step in adaptive immunity. Toll like receptors 2 and 4 on antigen presenting cells and phagocytes and nucleotide binding oligomerization domain 1 (NOD 1) in the cytoplasm have been found to play a critical role in the mucosal defense against polysaccharide encapsulated *Haemophilus influenzae*, wheras the protection against non-capsulated *HI* did not (112).

At the mucosal surface the pneumococcus can decrease the viscosity of the mucus with neuraminadases leading to the exposure of surface receptors that can interact with the pneumococcal surface-associated proteins (reviewed in (113)). Secondary to cytokine stimulation the epithelial cell upregulates PAF receptors to which the pneumococcus increases the affinity through its cell-wall phosphocholine. In addition the CbpA/PspC, can bind directly to the polymeric Ig receptor (pIgR) on the epithelial cell and pneumococcal IgA1 protease can cleave opsonising IgA which increases migration through the mucosa. Altogether, the interactions between the pneumococcus and the epithelium can facilitate pneumococcal invasion and lead to systemic infection (84). The increased invasiveness of pneumococci following influenza infection may be explained by neuraminidase activity of the viruses contributing to enhanced adherence of the pneumococci (114, 115). Surface associated pneumococcal proteins are now increasingly investigated as possible vaccine candidates. Some of the pneumococcal proteins are present on a vast majority of pneumococcal strains belonging to most of the known serotypes and such a vaccine would have the potential of decreasing colonization as well as systemic disease across all serotypes (116-118).

Systemic immune responses.

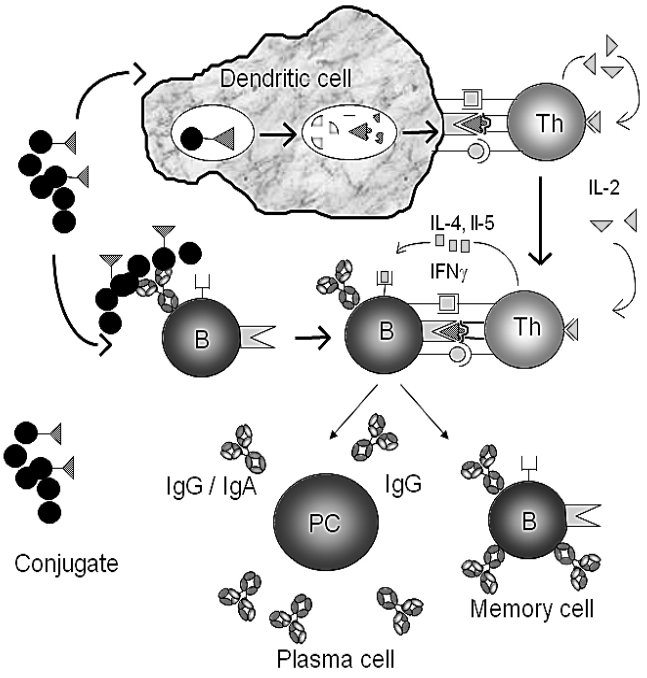
If the pneumococcus succeeds in evading the mucosal defence the systemic immune response takes over with opsonization and phagocytosis of the pathogen. The host defense against encapsulated bacteria depends on humoral immunity with antigen-specific antibodies, complement activation and phagocytic killing or lyses of gram-positive or gram negative bacteria, respectively. The humoral immune responses are divided into thymus dependent (TD) or thymus independent (TI) antibody responses depending on whether the antigen is presented in the major histocompatability complex class II (MHC-II) on antigen presenting cells (119).

TD proteins antigens and peptides derived from them associate with the MHC-II, leading to activation of T-helper cells inducing isotype switching and generation of immunological memory. Purified polysaccharides such as PPS stimulate B-cells in a T-cell independent manner. In infants and young children this leads to IgM antibody production that does not progress to isotype switching, somatic hyper mutation, affinity maturation or memory generation (119-121). In adults, a polysaccharide-specific IgM+ memory B cells, originating in the marginal zone of the spleen, have been described as a responsive cell type to the PPS and are thought to take part in the natural immunity to the pneumococcus. Children start to respond to PPS around 2 years of age, coinciding with the occurrence of the marginal zone B cells in the spleen (122, 123). Studies have also indicated that besides being not presented in the MHC II the polysaccharide immune response may differ due to the nature of the B cell antigen receptor signaling of the responding B cell subpopulations (124). In a study on SCID mice transplanted with human B lymphocyte subsets and then immunized with heat-inactivated streptococcus or with PPV23, IgM anti-polysaccharide and anti-protein antibody responses from IgM memory B lymphocytes were observed. IgG anti-polysaccharide and anti-protein responses were also observed from switched memory B lymphocytes. In addition, IgM memory B cells elicited an IgG anti-polysaccharide and anti-protein response indicating versatile role of IgM memory B cells in T-independent and T-dependent immune responses (125). Mouse studies have further shown that immunization with PCV induces carrier-specific T cell responses that increase with age and determine the levels of PPS-

specific Ab elicited. A weak and Th2-biased response was observed in neonatal mice while infant mice showed a mixed Th1-Th2 response, as observed in adults (126).

Serotype polysaccharide specific IgG and complement confer protection against the given pneumococcal serotype. Newborns and infants up to the age of 2 years are unable to produce IgG antibodies to bacterial capsular polysaccharides (127). Consequently children up to the age of 2 years have an increased susceptibility for infections with encapsulated bacteria such as pneumococcus and *Haemophilus influenzae* type b and *Neisseria meningiditis.* The polysaccharides have been rendered immunogenic by direct covalent binding to immunogenic proteins (128). These polysaccharide-protein conjugates are taken up by antigen presenting cells that present the protein moiety in the MHC II molecule and induce clonal expansion of protein specific T helper cells (Figure 1).

Figure 1. B- and T- cell collaboration in immune response to PCV



The figure is adapted from G. Vidarsson, PhD Thesis, University Medical Centre Utrecht, The Netherlands.

Polysaccharide specific B-cells which take up the same conjugate will by antigen presenting function also stimulate protein specific T-cells that in turn with CD40-CD40L and B7-CD28 interaction and secretion of the necessary cytokines (IL4, IL6) push the B-

cell into isotype switching and further development into plasma cells or memory B cells (119, 129, 130).

Depending on the antigen the IgG responses are of variable IgG1 and IgG2 composition. The natural adult immune response to polysaccharide antigen is mainly of the IgG2 subclass (131), giving a ratio of IgG1:IgG2 < 1 while the response to protein antigen is mainly of the IgG1 subclass leading to ratio of > 1 (132). The infant and toddler IgG responses are mostly of IgG1 subclass giving the IgG1:IgG2 ratio >1 since infants are unable to make IgG2 (133-135).

Correlates of protection.

The early method of evaluating anti-pneumococcal polysaccharide antibodies was a radioimmunoassay (RIA) which measured total antibody including all isotypes (136). Vaccine-induced total antibodies of 250 to 300 ng of Ab μg/ml were estimated to confer protection against pneumococcal infections in adults at risk (137). The current method of measuring pneumococcal antibodies is by enzyme-linked-immunosorbent assay (ELISA) in which isotype and subclass specific data can be provided (138, 139). Luminex type methods are also being used allowing simultaneous measurements of antibodies to several serotype in small sample volumes (90, 140). Expert consultation sponsored by WHO on serologic criteria for the evaluation of new PCVs have led to published guidelines for quantitating serotype-specific IgG antibodies to pneumococcus in human sera (141, 142). Information on invasive disease in the PCV efficacy trials (143-145) led to a proposed protective level against IPD of 0.35 μg of IgG anticapsular antibody/mL. Similarly, correlate of protection for AOM was found to be about 10 times higher for four serotypes, 6A, 6B, 19F and 23F in the Finnish Otitis Media efficacy trial (FinOM) where the relationship was serotype specific, correlating the best for serotype 23F and worst for 19F (146). In mice immunized intranasally with pneumococcal serotype 1-TT conjugate and challenged intranasally with same serotype, a 10 fold higher level was required to protect against lung infection than against blood infection and decreases in pneumococcal density correlated to pneumococcal IgG andibody levels (147). An inverse relationship has been described between serotype specific serum IgG levels to serotypes 6A, 9V, 14, 19F and 23F and nasopharyngeal acquisition of same

serotypes which indicates a role of serum antibodies in mucosal protection (148, 149). Similar findings for natural exposure to serotype 14 and acquisition were found with the level of 5μg/mL as correlating with protection (150).

Other *in vitro* correlates of protection include the opsonophagocytosis activity (OPA). Opsonophagocytosis is the primary defense mechanism against pneumococcus and the goal of any pneumococcal vaccine is to elicit opsonizing antibodies. The OPA methods have been evolving for the last 10 years using either uptake (151, 152) or killing (153) of the bacteria by blood neutrophils or phagocytic cell lines. OPA has been applied in many vaccination studies and collaborative efforts have led to the development of a consensus OPA method (154) that, however, is still being developed (155-157). Opsonophagocytosis has been shown to correlate strongly with serotype specific antibody levels (151, 158, 159, 160) and has been suggested as an indicator for protective capacity of antibodies induced by new PCVs when bridging between phase II trials and efficacy trials (161). OPA has even better predictive values for vaccine efficacy than IgG concentration only. This was demonstrated in the FinOM efficacy trial where serotype 19F induced high levels of IgG but low OPA values which reflected lower efficacy against AOM (153, 161). Correlation between OPA and serotype specific antibody levels has been enhanced by preadsorption of non-functional antibodies against the cell wall polysaccharide (CWPS) in the ELISA (151). Further, by neutralization of the test sera with serotype 22F, the ELISA detects antibodies with higher serotype specificity as has been shown with increased correlation between OPA and IgG ELISA results in post-vaccination adult sera (162). The preadsorption with 22F polysaccharide is especially important in individuals who have been vaccinated with PPV23 or been naturally exposed to pneumococci but plays lesser role in post vaccination infant sera. This may, however, be important in highly endemic areas.

Pneumococcal vaccines.

To date there are two types of licensed pneumococcal vaccines, PPV and PCV.The dramatic decrease in invasive *Haemophilus influenzae b* disease after the polysaccharide conjugate vaccination (128, 163) set the level of what to aim for in pneumococcal

conjugate vaccination development. The method of covalent binding of bacterial polysaccharides to protein carriers converted the TI immune response to a TD one with subsequent isotype switching and memory generation. The 23 valent pneumococcal polysaccharide (PPV23) a 7-valent (PCV7) and a 10 valent conjugate vaccines just registered in 2009 are now the only licenced pneumococcal vaccines.

The pneumococcal polysaccharide vaccine (PPV23)

When antibiotics failed to eliminate pneumococcal disease in 1950s a new interest was gained in vaccine development. The current 23 valent PPV (PPV23), developed from a 14-valent PPV (164), has been registered since 1983. It contains 25µg of each of the 23 serotypes of pneumococcal polysaccharide (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33). The current recommendations for its use include guidelines for the immunization of adults and children over 2-years of age who are at high risk for IPD, of the elderly ≥ 65 years and individuals who have a HIV infection (23). It soon became clear that PPV23 vaccine was not efficacious among infants and young children when evaluated against acute lower respiratory tract infection although some protection was observed in children over 17 months of age (164) and there was no decrease in mortality in this age group (165, 166). In a Finnish field trial on PPV14 against recurrent otitis media, no protection was observed in infants less than 6 months of age and no protection to serogroup 6 up to 6 years of age although a significant reduction was observed in older children to other vaccine serotypes (167). Post licensure epidemiological studies have shown some effectiveness of PPV23 vaccine in preventing IPD in the elderly or adults with increased susceptibility to pneumococcal infection although some randomized controlled trials failed to demonstrate vaccine efficacy for invasive pneumococcal infection, but these were mostly underpowered (168, 169). PPV14 and PPV23 vaccines were 56% effective against IPD in a case control study in gold-miners in South Africa (170). Meta-analyses have shown that PPV23 offers some protection against IPD in the general elderly population but optimal prevention was not obtained (171, 172). In a recent large scale population study in Sweden where elderly people were vaccinated with PPV23 the incidence of vaccine-type IPD declined significantly during the study period (1997-2001), from 50 to 28.9/100,000

pr year compared to a community where no vaccination campaign was performed (173). The PPV23 can prevent some IPD in adults and older children but does not induce protective immune responses in infants and young children who are at the highest risk.

Pneumococcal conjugate vaccines (PCV).

One PCV has been licensed since 2000, Prevnar®/Prevenar® (Wyeth), which contains polysaccharides from seven pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F and 23F), conjugated to the cross reacting material (CRM_{197}), a non-toxic mutant of diphtheria toxin and contains the adjuvant aluminum phosphate (PCV7). The optimal dose of PPS for the CRM_{197} carrier was found to be 2µg of capsular PPS of the serotypes 4, 9V, 14, 19F, and 23F, 4µg of type 6B PPS, and 2µg of type 18C oligosaccharide – conjugated to CRM_{197} (174-176). In an investigational 9-valent PCV- $CRM_{197,}$ 2µg of each serotypes 1 and 5, were added to improve the coverage in the developing countries (177, 178).

An 11-valent PCV-PD (GlaxoSmithKline) contains 1µg each of capsular PPS of serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F conjugated individually to a recombinant nonlipidated form of protein D (PD), a cell surface lipoprotein of *H. influenzae.* PCV11-PD was a developmental formulation for Synflorix® (GSK) a 10-valent PCV (PHiD-CV) which includes 1 µg of serotypes 1, 5, 6B, 7F, 9V, 14 and 23F and 3 µg serotype 4 conjugated to protein D (PD) and 3 µg 18C conjugated to Tetanus toxoid (TT) and 3 µg 19F conjugated to diphtheria toxoid (DT)[1]. The Syflorix® was licenced in Canada in January 2009 and is awaiting approval in Europe. Studies on the developemental PCV11-PD involved 11 serotypes (179); serotype 3 is not included in the final PHiD-CV formulation.

A developemental 7-valent PCV, conjugated to outer membrane complex of N. meningiditis (PCV7-OMPC) containied four serotypes (6B, 14, 19F and 23F) (180) and

[1] Publications in press. Personal communications from William Hausdorff, PhD, Director, Epidemiology & Scientific Strategy, GlaxoSmithKline Biologicals, Site de Wavre-Nord/W35, Avenue Fleming 20, 1300 Wavre, Belgium.

then seven serotypes; 6B, (5 μg), 23F (3μg), 18C and 19F (2 μg each), 9V (1.5μg) and 4 and 14 (1 μg each) each conjugated to OMPC. This vaccine was investigated in phase I – III trials, one efficacy trial on AOM (181) but was not registered.

The other PCV formulation which was developed but did not suvive registration was an 11-valent PCV with serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F individually conjugated to DT or TT mixed carriers (sanofi pasteur). This formulation was developed based on an earlier monovalent 6B-TT vaccine (Conjugate prepared by Dr. J.B. Robbins and Dr. R. Schneersson, NICHD/NIH, Bethesda, US (182), Papers I and II). Investigational vaccines of different valencies were investigated in phase I – III clinical trials as four-valent (183-185), eight-valent single carrier formulation ((186) and Paper III) and 11- valent mixed carrier formulations with (PCV11-F3-alum) (187-190) or without aluminum hydroxide adjuvant (PCV11-F3) ((187, 190, 191) and unpublished data from Trial IV). In Trial III and IV, supporting this PhD thesis, we investigated a formulation containing both DT and TT carriers for four of the 11 serotypes (PCV11 – F3bis).

A list of studies on safety and immunogenicity performed in infants using PCV formulations containing five carrier proteins, CRM_{197}, OMPC, DT, TT or Protein D is presented in Appendix I.

Safety of PCVs.

The immunogenicity and safety of PCVs have been investigated extensively. The PCVs differ in terms of number of serotypes, polysaccharide dose, carrier proteins, whether they contain an adjuvant or not. They have been investigated when administered at different schedules and number of primary doses, with or without a booster dose and with different concomitant routine infant vaccines.

When studies are performed in infants the trial vaccines have already passed safety and immunogenicity studies in adults and toddlers.

In a recent systematic review of the safety of PCV7, 42 published articles and two abstracts were identified from searches of the PubMed, Cochrane Collaboration databases, bibliographic references and expert consultations within the field (192). The

review indicated the safety of Prevnar usually with mild local side effects and mild fever but with increasing rate with number of injections (143, 193-196) with the highest rate after the toddler dose. This trend was not observed for more severe reactions (195-198). According to post-marketing studies Prevnar vaccine is considered to be as safe as other routinely used vaccines (199). The Vaccine Adverse Event Reporting System (VAERS) is a cooperative program of the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) in the USA on vaccine safety (200). VAERS is a post-marketing safety surveillance program, collecting information about adverse events (possible side effects) that occur after the administration of USA licensed vaccines. VAERS data are derived from a passive surveillance system and represent unverified reports of health events, both minor and serious. According to VAERS, the post-marketing surveillance for PCV-7 reported similar rate of serious adverse reactions as reported for other vaccines (199).

Some increases have been reported in respiratory symptoms after PCV. A transient increases in respiratory infections, mainly due to Respiratory Syncytical Virus was observed in the first week after PCV9 vaccination in South Africa and also some increase in reactive airway disease compared to the placebo group (144). In the Gambia more total outpatient consultations were observed after the first dose of PCV9 but not after subsequent doses (160). In an efficacy trial using PCV11-DT/TT in the Philippines a small but significant increase in all severe adverse events (SAE) was observed after the first doses of PCV vaccination, partly due to increase in pneumonia. SAE were also increased after the second dose (191).

The PCV11-PD vaccine was safe when administered with routine infant vaccinations (179, 201). Safety of the PCV11-PD vaccine has been published up to two years after vaccination, demonstrating a safe vaccine (179).

Outer membrane complex of N. meningiditis (OMPC) conjugate, was investigated and found to be safe and immunogenic in infants (180, 202-204).

PCV-TT and PCV-DT vaccines were investigated as a monovalent (Paper I), four-valent (183-185) and eight-valent single carrier formulation ((186) Paper III) and in an 11-valent mixed carrier formulations with (PCV11-F3-alum) (187-190) or without

aluminum hydroxide adjuvant (PCV11-F3) ((187, 190) and unpublished data from Trial IV). These trials showed that the vaccines were safe when administered to infants with routine vaccinations (205-207).

Immunogenicity.

Measurement of serotype specific antibody concentration by ELISA before and 4 weeks after primary series vaccinations as well as booster vaccination has become the standardized method of evaluating immunogenicity of PCVs (141, 142). In addition to measurements of antibody concentration, the functional capacity as evaluated by OPA and quality of antibodies by avidity measurements has been used to estimate protective capacity of investigational vaccines (142, 154). Induction of immunological memory has been evaluated by boosting with PPV23 booster to demonstrate a memory response to the native PPS and avidity maturation.

The PCV7- CRM_{197} (from now on called PCV7) was shown to be immunogenic in infants (194, 208-210) and was tested in three efficacy trials, one against IPD (143) and two against AOM (145, 193). In general, antibody responses are lowest to serotypes 6B and 23F and highest to serotypes 14 and 19F. CRM_{197} was first investigated in a 5 serotypes formulation (6B, 14, 18C, 19F, 23F) (174-176, 211), demonstrating safety and significant serotype specific IgG responses. By adding serotypes 4 and 9V a 7-valent formulation was formed; the PCV7 which similarly proved safe and immunogenic in infants (143, 194, 210, 212, 213). The same carrier was used for a 9-valent vaccine by adding serotypes 1 and 5 which are important pathogens in the developing world. That 9-valent vaccine demonstrated similarly good safety and immunogenicity (177, 178) and was used in two efficacy trials in Africa (160, 214, 215). PCV7- CRM_{197} induced antibodies have been shown to be functional as evaluated by OPA (153, 216) and primed for robust memory responses to a PPV23 booster (153, 186, 217-219). Eighteen months after immunization significant serotype specific IgG, IgG1 and IgG2 rises were observed 10 days after PPV23 booster coinciding with avidity maturation and opsonophagocytic activity (216).

PCV-PD used in the AOM efficacy trial in the Czech Republic (220) showed significant IgG responses to all 11 serotypes in the vaccine when given at 2, 4 and 6 months with a booster at 12 – 15 months (179). Pre booster the antibody levels were significantly higher than in unvaccinated controls and a PPV23 booster dose at 12 – 15 months of age induced a significant memory responses, indicating priming and existing B-cell memory. A booster dose with the PPV23 induced higher IgG levels than the PCV11-PD booster which has been described for other PCVs as well (181, 202, 207, 221). One dose of PCV-PD given at 12-15 months induced significant primary responses for all but serotypes 6B and 23F (222), demonstrating the poor immunogenicity of these two serotypes also shown by other vaccines.

The PCV7-OMPC, co-investigated with PCV-CRM_{197} in the AOM efficacy trial in Finland (FinOM Vaccine Trial) (146, 153), was shown to be immunogenic in infants but with different serotype specific kinetics of antibody responses and avidity maturation than PCV7 (223). The concentration of IgG against serotypes 6B, 19F, and 23F declined faster after the third and fourth doses of PCV7- CRM_{197} than after the PCV7-OMPC, but PCV-OMPC did not elicit higher responses after the later doses than after the first dose, suggesting a lack of immunological memory. For both PCVs, the mean avidity index (AI) of anti-6B and -23F, increased during the follow-up which was not observed for anti-19F. The lack of the avidity maturation may have had something to do with the low protection against serotype 19F observed in the FinOM Vaccine Trial (146). Due to the lower immunogenicity profile of the OMPC conjugate vaccine and apparent lack of booster responses as compared to PCV7- CRM_{197}, this PCV was not developed further and has not been registered.

The 4-valent formulations of the PCV-DT and PCV-TT vaccines were immunogenic, providing significant IgG responses (184, 207, 224) that were functional. These studies investigated the optimal polysaccharide dose for each serotype (184, 224), PCV-TT demonstrating highest response to the lowest dose tested, 1µg/serotype. On the other hand the highest dose of the DT conjugate tested (10 µg/serotype) gave the highest IgG response. For both formulations the 1µg/serotype dose gave the highest booster responses to PPV23 vaccine at 12 months. The vaccines were further developed into 8-valent formulations (186). As part of the research for this PhD thesis we searched for an

optimal carrier for each serotype when we compared the immunogenicity of DT and TT conjugates for eight serotypes (Paper 3, Trial II). The aim was to develop an 11- valent vaccine that would carry appropriate serotype composition for use in infants in both developed and developing countries. Based on the preceding trials, three 11-valent formulations were compared. PCV11-F3 contained 1μg pr serotype 1, 4, 5, 7F, 9V, 19F and 23F conjugated to TT and 3μg pr serotype 3, 14, 18C and 10μg 6B conjugated to DT. The second formulation (PCV11-F3-alum) had the same serotype composition but also aluminium hydroxide as an adjuvant. Although DT or TT carriers were selected for each serotype based on the best immune responses, improvements of primary immune responses were still searched for serotypes 6B, 9V, 18C and 23F. In order to explore if the T cell help was limiting we investigated and compared with the PCV11-F3 the third 11-valent formulation, PCV11-F3bis, which contained same serotypes but both DT and TT carriers for each of the four poorly immunnogenic serotypes, aiming at to answer if providing two carrier proteins for each serotype, thus increasing the T-cell help, would improve their immunogenicity (Trial III and IV; unpublished data).

The comparison of PCV11-F3-alum and PCV11-F3 formulations in Filipino infants showed that both vaccines were immunogenic. The PCV11-F3-alum induced higher GMCs than PCV11-F3 but both induced high levels of serotype specific antibodies (188, 189) and memory responses at 9 or 12 months and avidity maturation (189, 190). Of interest is that the IgG antibody responses to the tetanus-conjugated polysaccharides were considerably higher in the Filipino than in the Finnish or Israeli infants. The authors give the possible explanation that these differences may be a result of the priming effect of tetanus toxoid given to pregnant women, early pneumococcal nasopharyngeal acquisition and genetic differences among populations. In the PCV11-F3/F3-alum trials in the Philippines maternal TT antibodies were not found to inhibit the antibody responses to the PCV11-F3-alum polysaccharides when administered at 6, 10 and 14 weeks of age (187). The infant responses to the PCV11-F3/PCV11-F3-alum were not inhibited by higher maternally derived PPS antibodies if compared to infants in Finland (187) which is in contrast to what was shown in Alaska where maternal antibodies were associated with a reduced infant response to first two doses of PCV7-CRM_{197} given at 2, 4 and 6 months but did not interfere with memory responses to 4^{th}

dose at 12 months (225). On the basis of the above trials the PCV11-F3 vaccine was selected for an efficacy trial against pneumonia in the Philippines (191).

Vaccine interferences.

Co-administered conjugate vaccines with the same protein carrier as in the PCV can cause interference, resulting in decrease in antibody responses to either or both conjugated polysaccharides and to the carrier protein. Carrier mediated interactions have been described where concomitant administration of PCV-TT, Hib-TT (PRP-T) and Dipththeria/Tetanus/whole cell Pertussis (DTwP) resulted in diminished responses to TT and Hib (184, 207, 226). In Israel, a study comparing PCV11-mixed TT/DT carrier conjugates with regular concomitant vaccines containing DTwP-IPV-PRP-T with PCV11-DT/TT with concomitant vaccines containing DTaP-IPV-PRP-T, resulted in significantly lower responses to TT and the TT-conjugated serotypes in the group receiving the Dipththeria/Tetanus/acellular Pertussis (DTaP)-IPV-PRP-T (227). Thus, the change from whole cell to acellular pertussis vaccine resulted in diminished antibody responses to the tetanus protein and the TT-conjugated serotypes in the PCV11. In one of the trials included this thesis, interference between DT-conjugated PPS and Hib was studied (Trial II).

Other studies did not show similar reduction when acellular pertussis (aP) was administered with PCV7 (197, 228) but a carrier mediated suppression has also been described with CRM_{197} conjugate vaccines (210, 229) although an enhancement has also been described in antibody responses to other vaccines conjugated to the same carrier (212).

Reduced number of infant doses of PCVs

The PCV7 is registered for 3 doses between 2 and 6 months of age with at minimum 4 weeks interval and a booster during 2nd year of life. Other schedules have now been allowed in order to make PCV immunization possible with other infant immunizations.

In the developing countries the PCV has been administered with infant vaccinations at enhanced schedule at 6, 10 and 14 weeks in order to induce earlier immunity to infection (The Expanded Program on Immunization). Studies on immunogenicity of PCV9 according to that schedule have shown significant antibody production and effective priming (177). This schedule has also been investigated with DT and TT PCVs showing significant immunogenicity (189, 230). Immunogenicity of PCV7 administered in two primary doses has been investigated in nonrandomized studies (217, 231, 232) showing antibody production of similar magnitude and avidity maturation indicating memory development (217) but also of lower primary response to serotypes 6B and 23F although memory responses were comparable indicating similar priming (233). One aim of the work for this PhD thesis was to answer in a prospective randomized design if a PCV can be administered in two infant doses without compromising its immunogenicity. We investigated the immunogenicity of PCV9 combined with a Meningococcal C conjugate vaccine when given in two or three primary dose schedules (Trial V, Paper V).

A booster PVC dose in the second year of life is a part of recommended vaccination schedule in the developed countries. The antibody levels after the primary schedule decrease rapidly although in most studies they remain higher at time of booster than in unimmunized controls. The decreased antibody levels coincide with the age when nasopharyngeal colonization is most prevalent, increasing the risk of pneumococcal disease. Evidence from UK on *Haemophilus influenzae* type b and serogroup C meningococcal conjugate vaccines indicate that a booster vaccination with a conjugate vaccine in the second year of life is necessary to maintain immunity (234, 235). In the developing countries where pneumococcal colonization and prevalence of pneumococcal disease is very high the natural exposure may be sufficient to provide a booster effect in healthy children (215). However, in HIV infected children the antibody levels were not maintained and a booster dose may be needed to keep up protection.

The number of primary PCV doses required to induce sufficient protective immune responses in infants has not been extensively investigated. However, vaccinations with two primary doses and a booster dose at 12 or 13 months of age have already been implemented in the UK(236), Norway (237), Denmark (238), Sweden (239), Belgium and Swizerland (240).

Immunological memory

Long term protection based on immunological memory generated by PCV is the optimal goal for PCV vaccination in infancy. For most pneumococcal serotypes 2 – 3 primary vaccinations are required to induce significant IgG responses as measured four weeks after last primary dose. The antibody levels generated by primary immunizations wane rapidly (210, 213). In infants immunized with PCV9 at 6, 10 and 14 weeks of age, serotype specific IgG levels were still significantly higher than in controls for all nine serotypes at time of booster at 18 months (241). In a long term follow-up study on children who were vaccinated with PCV9 at 6, 10 and 14 weeks without a booster in South Africa, the PCV9 serotype specific IgG levels were higher in vaccinated HIV negative vaccinees than the placebo control group. In the HIV negative group, 44% to 68% had IgG level > 0.35μg/mL and 19% to 81% in the HIV positive subjects (215). Using conjugate vaccine against Meningococcus C (MnCC) it was shown in a study from the UK that the magnitude of primary IgG responses and number of memory B cells after the primary vaccinations, correlated with the persistence of functional antibodies at 12 months of age (242). In the Czech Republic, using the PCV-PD vaccine, antibody levels to all 11 serotypes at 4 years of age were higher in the children vaccinated with PCV-PD at 3, 4, 5 and 12-15 month than in unvaccinated controls (243).

In the Gambia the memory responses in children who received 5-valent PCV-CRM_{197} at 2, 3 and 4 months or 2 and 4 months of age were investigated by measuring IgG responses before and 10 days after PPV23 booster vaccination at 2 years of age (216). Before the booster the vaccinees had significantly higher antibody concentrations compared with nonvaccinated controls, whether primed with two or three PCV doses and significant responses to the PPV23 in 10 days that were not observed in the controls. Children previously vaccinated with 4 doses of PCV-PD in the Czech Republic and boosted with PPV23 in the forth year of live showed significant IgG responses to all vaccine serotypes in 14 days (243)

The memory B cells provide enhanced immunity in antigen-dependent fashion upon exposure to the antigen (244) and may also be capable of generating plasma cells in an antigen-independent fashion, maintaining low levels of circulating antibodies (245).

In three of the studies included in this thesis we investigate the potential of PCVs to induce immunological memory by measuring the kinetic of IgG responses 7 days after booster vaccination (Trial II, III and V; Paper V and unpublished data).

Hyporesponsiveness

Several indications of polysaccharide administration causing hyporesponsiveness to conjugate vaccines have been reported. A Meningococcus C polysaccharide (MCPS) toddler dose following MnCC vaccination in infancy in the Gambia was shown to induce immunological hyporesponsiveness to subsequent MCPS challenge at 5 years of age (246). Thus, the MCPS challenge at 2 years compromized subsequent memory responses. Another study from The Gambia, investigating immunological memory to MenC and MenA, showed that when children previously vaccinated with two doses of MCPS vaccine were challenged with MCPS at 2 years they had significantly lower MenC antibody levels than children receiving only one dose before 6 months of age or nonvaccinated controls (247).

Full dose PPV23 vaccine has been used for booster vaccination following vaccinations with PCV vaccine to demonstrate existence of memory indicating successful priming by the PCV. With broadening the coverage by using PPV23 booster following PCV priming, theoretically more diseases may possibly be prevented in populations with high prevalence of pneumococcal diseases. This is the current recommendation for vaccination in populations with high incidence IPD (248) and in populations with increased susceptibility for IPD (23). In a recent study from Greece, the effect of PPV23 on immunological priming induced by PCV7 was investigated in 30 asplenic subjects with β-thalassemia (aged 12 to 41 years) grouped according to number of PPV23 doses received the preceding 2 to 5 years. Twenty three PPV23 naïve patients with Thalassemia served as controls (249). The patients were immunized one month apart with two doses of PCV7 or with PCV7 and PPV23, followed with PPV23 12 months later and IgG to five serotypes measured at each time point. The PCV/PPV23 combination did not affect PCV priming as both schedules resulted in similar antibody responses, indicating that this was a safe schedule in adults. However, three out of five

serotypes showed inferior priming in patients who had previously received two or more PPV23 vaccinations compared with the 23 aged-matched PPV23-naïve patients with β – thalassemia while patients who received only one previous PPV23 vaccination responded similarly to the controls. Evidences for hyporesponsiveness and possible mechanisms were recently reviewed in (250).

The possible immuno compromizing effect of PPV23 vaccination on memory B-cells generated by PCV vaccination has not been fully investigated in children. In one of the studies supporting this thesis we investigate in 7 year old children if a PPV23 booster at 13 months compromises immunological memory generated by priming vaccinations with PCV-TT in infancy (Amended study to Trial II, unpublished data).

Groups with increased susceptibility for IPD.

The highest incidence and highest mortality due to IPD is in the very young (22), at which age, the immune system cannot respond to TI antigens, and in the elderly when immunity to pneumococcus wanes (251, 252). Increasing risk for IPD is found in elderly persons who have co–morbidity, including diabetes mellitus, chronic heart disease, chronic lung disease, alcoholic abuse and cancer (253). Splenectomized patients are at risk for overwhelming invasive infection by encapsulated bacteria, especially *S. pneumoniae* (254, 255). This includes patients with hemoglobulinopathies such as sickle cell disease and thalassemia, patients who have had malignancy such as Hodgkin's lymphoma or have undergone splenectomy to solve thrombocytopenia or due to trauma. The regular use of PPV23 vaccine has been recommended for those patients (256, 257) but a combined schedule with PCV7 followed by PPV23 has been shown to induce higher antibody levels to the vaccine serotypes (258) which now is the current recommendation in sickle cell disease. The incidence of IPD in sickle cell disease has decreased significantly since PCV7 was introduced in general vaccination in the USA (259). By using PCV in splenectomized patients the T-cell dependent responses can take place in germinal centres outside the spleen, thus bypassing the requirements for functional splenic marginal zone.

Patients with HIV infection have defective antibody responses and are at greatly increased risk for IPD (260). PCV vaccinations of HIV infected individuals have recently been shown to be highly efficacious (261, 262).

The risk for IPD is high in patients undergoing bone marrow transplantation (263) or solid organ transplantation (264) in whom immunosuppressive therapy plays a big role.

Decreased or no capacity to produce antibodies to polysaccharides is a well recognized condition and may be as common as one in every 10 adults (265, 266). Studies have shown that infection-prone patients that are non-responsive to polysaccharides, i.e. have specific antibody deficiency, can mount antibodies to PCV vaccine although they respond with significantly lower antibody responses compared to normal controls but higher than to PPV23 vaccine (267).

Efficacy of PCV

Efficacy of PCV has been investigated in infants against IPD, pneumonia, AOM and nasopharyngeal colonization.

Efficacy trials against IPD and pneumonia

Five large-scale PCV efficacy trials with IPD and/or pneumonia as an endpoint have been conducted (summarized in Table 2 using the PCV-CRM_{197} in four (143, 145, 272, 214) and PCV-DT/TT in one (191).

In the Northern California Kaiser Permanente (NCKP) efficacy trial 37.868 infants were recruited. The PCV7 was used in a individually randomization design, concomitantly with routine infant vaccinations at 2, 4, 6 and 12-15 months in 18.927 infants and in the placebo arm 18.941 infants received Meningococcus C CRM_{197} conjugate vaccine (MnCC) (143). The efficacy against VT IPD according to intent to treat analysis was 94%. In the initial published report the effect against clinical pneumonia was 10.7% and 12.2% if with any chest radiography (268). Later, by using radiography evaluation according to WHO recommendation the efficacy against first episode of pneumonia was

26% for intent-to-treat and 30% for per protocol (269). No replacement disease was observed from NVT pneumococci (270).

In USA the PCV7 was used in an efficacy trial in 38 communities of American Indians with high risk for IPD. The communities were individually randomized to receive either PCV7 or MnCC at 2, 4, 6 and 12 – 15 months. A catch-up was given with two doses from 7 – 12 months and a booster and two doses from 12 – 24 months. Out of 8292 children enrolled less that 2 years of age, 4.165 received the PCV7 and 3.926 MnCC placebo. The primary outcome, VT IPD efficacy was 83% in the children immunized within 7 months and 86,4% if less than 24 months. No difference was in NVT IPD (145).

In South-Africa, PCV9 was used in an efficacy trial with individual randomization to receive PCV9 or true placebo at 6, 10 and 14 weeks of age and no booster. Both HIV-infected and uninfected were recruited but analyzed separately (214). Total of 19.922 children were vaccinated with PCV9 and 19.914 with true placebo. In each arm 1.289 and 1.288 children were HIV-infected, respectively. The first evaluation at 2.3 years of follow-up, showed efficacy against VT IPD of 83% and 65% in HIV negative and HIV-positive, respectively. A second analysis of efficacy was done after 6.16 years follow-up period showing efficacy against VT IPD of 77% and 38.8% for HIV negative and positive, respectively (215).The efficacy against first episode of radiologically confirmed pneumonia was 20% and 13% in HIV negative and positive children, respectively after 2.3 years. Thus, in this long term follow up efficacy study after 3 primary doses without a booster, a similar protection was observed in the HIV negative population after 6 years but a significant reduction was observed in the HIV infected population, from 65% to 38.8% ($p<0.0001$). Due to nonsignificant increase in NVT in the HIV negative population the overall reduction in IPD was almost halved (from 75 to 38 cases per 100.000).

In the Gambia, the PCV9 was used in an efficacy trial against IPD and pneumonia (272). The trial was individually randomized into 8718 infants receiving PCV9 and 8719 receiving true placebo with three primary doses after 6 weeks of age. The PCV9 was

37% effective after median follow up of 25 months and 77% effective against any VT IPD and 16% against mortality (272).

In the Philippines, the 11-valent mixed DT and TT carrier PCV was used in a double blinded placebo control trial where 6013 infants received the PCV11-DT/TT in three primary doses between 6 weeks and 6 months without a booster and 6018 received a true placebo. After two years, the vaccine showed 22.9% efficacy (p=0.06), most significant in children less than 12 months of age with 34% efficacy (p=0.02) (191).

Efficacy trials against AOM

Four efficacy trials have evaluated the efficacy against AOM (summarized in Table 3). Three used PCV7- CRM_{197} out of whom one used also seven valent PCV-OMPC (143, 181, 193, 271) and one using PCV-PD (220).

In the KPNC trial the efficacy of the PCV7 against AOM showed decreases in AOM visits by 8.9%, frequent AOM by 7% and ventilatory tube placement by 20.1% (64, 143).

In the FinOM study in Finland, two PCVs were randomized to be used in a double blind placebo controlled efficacy trial against AOM, PCV7 and PCV7-OMPC with Hepatitis B placebo (181, 193). The PCVs had 6% and -1% efficacy against any cause AOM for CRM_{197} and OMPC carrier, respectively, 34% and 25% for any type of pneumococcus AOM, 57% and 56% for VT AOM, 51% and -5% for VRT AOM and -33% and -27% for NVT AOM, for CRM_{197} and OMPC carrier, respectively. The serotype specific efficacy ranged from 25% for serotype 19F to 84% for 6B for PCV7- CRM_{197} and 37% for 19F to 82% for 9V for PCV-OMPC (193).

Table 2. Efficacy against IPD and pneumonia in infants.

Vaccine and schedules	Country	Number of PCV infants	Trial design	Outcome measure	% efficacy (CI)	Reference
PCV7-CRM_{197} 2,4,6 mo. primary 12 – 15 mo. booster	USA 23 Northern California Keiser Permanente medical centers	PCV: 18927 MnCC- CRM_{197}: 18.941	DBPC individual randomization	IPD caused by VTP First episode of radiological pneumonia	94% (80;98) 25.5% (6.5;40.7)	(143) Black et al. 2000 (269) Hansen et al 2006
PCV7-CRM_{197} Primary: 2, 4, 6 mo. Booster: 12 – 15 mo. Catch up at 7 – 11 mo: 2 doses and a booster Catch up at 12 – 24 mos: 2 doses with minimum 8 weeks in between.	USA 36 Navajo nation and 2 White Mountain Apache reservations	PCV: 4165 MnCC- CRM_{197}: 3.926	Group randomized	IPD	83% (22;96)	(145) O'Brien et al. 2003
PCV9-CRM_{197} HIV uninfected From 6 weeks of age 3 primary doses at least one month apart. No booster	South Africa, 21 clinics in Soweto	PCV: 18.633 True placebo: 18.626	DBPC individual randomization	IPD First radiolgically confirmed pneumonia IPD due to PRP	77% (19;93) 2007 83% (39;97) 2003 20% (2;35) 2003 67% (19;88) 2003	(214, 215) Klugman et al 2003 Madhi et al. 2007
PCV9-CRM_{197} HIV infected From 6 weeks of age 3 primary doses at least one month apart. No booster	South Africa, 21 clinics in Soweto	PCV: 1.289 True placebo: 1.288	DBPC, individual randomization	IPD	65% (24;86) 2003 28% (-22;58) 2007	(214, 215) Klugman et al 2003 Madhi et al. 2007
PCV9-CRM_{197} reconstituted in Hib-DTwP From 6 weeks of age 3 primary doses at least one month apart. No booster	The Gambia 15 fixed facilities and 110 outreach sites in rural Gambia	PCV: 8718 True placebo: 8.719	DBPC individual randomization	1) First episode of radiological pneumonia 2) Any cause VT IPD 3) Motality	1) 37% (27;45) 2) 77% (51;90) 3) 16% (3;28)	(272) Cutts et al. 2005
PCV-DT/TT (PCV11-F3: 1, 4, 5, 7F, 9V, 19F, 23F: 1µg pr serotype conjugated with Tetanus protein and serotype 3, 14, 18C: 3µg and 6B: 10µg pr serotype) Vaccinated at 6, 10 and 14 weeks of age, starting between 6 weeks and 6 months and minimum of 4 weeks between doses.	The Philippines Bohol	PCV: 6013 Placebo: 6018	DBPC Individual randomization	1. X-ray confirmed community-acquired pneumonia (CAP) 2. Clinical CAP 3. IPD with VT SAEs	Xr-CAP (PPA): 3-23 mo: : 22.9% (p=0.06) 3-11 mo: 34% (p=0.02) 12-23 mo:2.7% (p=0.88)	(191) Lucero et al. 2008

PCV7- CRM_{197}: Prevnar (Wyeth). Contains 7 serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated with CRM_{197} burðarprótein. Mo: Months of age. DBPC: Double blinded, placebo controlled. IPD: Invasive pneumococcal diseases. VTP: Vaccine type pneumococci,. PRP: Penicillin resistant pneumococci. CAP: Community aquired pneumonia. SAE: Severe adverse events. PPA: Per protocol analysis.

In Navajo and White Mountain Apache efficacy trial the PCV7 was used to vaccinate in a 2, 4, 6 and 12-15 schedule. Towards the end of the trial all clinical records from the vaccinees and the controls were analyzed, looking for any visits for AOM. The vaccine efficacy for any clinically diagnosed AOM was none (-0,4%) where the efficacy for severe OM was 5.1% and from draining ears the efficacy against VT pneumococci was 64% (271).

In the Czeck Republic efficacy trial the PCV11-PD was investigated in 2489 infants randomized against 2479 infants receiving Hepatitis A placebo. The PCV-PD efficacy against all AOM was 33.6%, against first episode VT AOM 52.6%, any episode VT AOM 57.6%, VRT AOM 65.6%, NVT was not significant. Efficacy against nontypeable *Haemophilus influenzae* (source of carrier protein) AOM was 35.3% (220).

Taken together the investigated PCVs have demonstrated significant efficacy against the VT IPD and pneumonia with decreased mortality and morbidity.

PCVs showed efficacy against AOM, as a significant reduction was observed in vaccine type infection in all the trials. The differences in epidemiology between the geographical areas where the trials were done may explain some of the differences observed between the results of the trials.

Table 3. List of PCV efficacy trials against AOM in infants.

Vaccine and schedules	Country	Number of PCV infants and controls	Trial design	Outcome measure	% efficacy (95%CI)	Ref. Year of start
PCV7- CRM_{197} **(Wyeth)** Primary: 2,4,6 mo. Booster: 12 – 15 mo.	USA 23 Northern California Keiser Permanente medical centers	PCV: 18927 MnCC- CRM_{197}: 18.941	DBPC individual randomization	1. AOM visits 2. Frequent AOM visits tympanostomy tube procedures	1. 7.8% (5.4-10.2%) 2. 10% reduction in the risk of 3 visits to a 26% reduction in the risk of 10 visits within a 6-month period 24% (12- 35%).	(64, 143) 1995 Black S et al Firman B et al.
PCV7- CRM_{197} **(Wyeth)** Primary: 2, 4, 6 mo. Booster: 12 – 15 mo.	Finland (FinOM trial)	PCV- CRM_{197}:831 HBV: 831	DBPC individual randomization	Culture-confirmed pneumococcal AOM episodes due to VT First episode due to VT, any serotype, clinical otitis episodes, OME	1. Any cause AOM 6% (–4 -16) 2. Any pn AOM 34% (21- 45) 3. VT pn 57% (44 - 67). 4. VRT pn 51% NVT pn increased by 33%	(193) 1995 Eskola J et al.
PCV7-OMPC (Merk) Primary: 2, 4, 6 mo. Booster 12 – 15 mo: PCV: 648 PPV23: 187 .	Finland (FinOM trial)	PCV-OMP: 835 HBV: 831	DBPC individual randomization	Culture-confirmed pneumococcal AOM episodes due to VT First episode due to VT, any serotype, clinical otitis episodes, OME	1. Any cause AOM 0% (–12-10) 2. Any pn AOM 25% (21- 45) 3. VT pn 56% (–44-66). 4. VRT pn - 5% (- 47 – 35) NVT pn -27% (-70 – 6)	(181) 2003 Kilpi T et al..
PCV7- CRM_{197} **(Wyeth)** Primary: 2, 4, 6 mo. Booster: 12 – 15 mo. Catch up at 7 – 11 mo: 2 doses and a booster Catch up at 12 – 24 mo: 2 doses with minimum 8 weeks in between	USA 36 Navajo nation and 2 White Mountain Apache reservations	PCV: 424 MnCC-CRM_{197}: 432	DBPC, group randomized,	1. Clinically diagnosed AOM 2. Severe OM 3. Vaccine serotype pneumococcal OM	1. -0.4% (-19.4 - 15.6) 2. 5.1% (-51.5 - 40.6) 3. 64% (-34% to 90%)	(271) 2008 O'Brien KL et al.
PCV11-PD (GlaxoSmithKline) Primary: 3, 4, 5 mo. Booster: 12 – 15 mo.	Czech Republic and Slovakia	PCV11-PD: 2455 Hepatitis A: 2452	DBPC individual randomization	1. First episode of culture confirmed VT pn AOM 2. First episode of culture confirmed non typable *Haemophilus influenzae* (NTHI) AOM, 3. Clinical episodes of AOM	1. All AOM: 33·6% (20·8–44·3) 2. Firs episode VT pn: 52·6% (35·0–65·5) 3. Any episode VT pn: 57·6% (41·4–69·3) 4. NTHI: 35·3% (1·8–57·4) 5. VRT: 65.6% (22.4-84.7) NVT: NS (p=0.766)	(220) 2000 Prymula R et al.

The table lists the five efficacy trials that have been performed against AO

Effectiveness, transmission and heard immunity

Following general introduction of PCV7 in the USA in 2000 a decline in rates of invasive pneumococcal disease has been observed (274). In the elderly the decline is accounted for by decreases in the number of cases of bacteremia and pneumonia, but not cases of meningitis (275). In addition to direct decrease in IPD among vaccinated infants and children (276) a decrease has been observed in pneumonia (277), medical visits due to otitis media (278) as well as meningitis (279). Besides the successful efficacy against pneumococcal diseases amongst the vaccinees an indirect efficacy has been observed in other age groups (274). A decrease in nasopharyngeal carriage which in turn results in less transmission and heard immunity most likely provides the explanation for this extensive heard effect from the vaccine (280, 281).

Replacement disease, need for new vaccines.

At same time as VT pneumococcal diseases have decreased an increase has been confirmed in NVT nasopharyngeal colonization (282) as well as NVT pneumococcal diseases which have increased significantly in IPD since PCV7 introduction in 2000 (283). The gain from the PCV7 vaccination with lives and morbidity spared still overweighs the threat of NVT disease. Still this stresses the need for further vaccine development against pneumococcal diseases. Two PCVs are in the pipeline, the 10-valent mixed PD, DT and TT carrier conjugate (PHiD-CV) already mentioned. The PHiD-CV contains serotypes 1, 5 and 7F in addition to the PCV7 serotypes which will increase the coverage for other geographical areas than USA. The PHiD-CV was registered in Canada in January 2009 and in Europe in March 2009 (Synflorix®). Still the PHiD-CV does not contain serotype 19A which is the main replacing serotype following PCV7 vaccination (284). The other vaccine that is being developed is the 13-valent CRM_{197} conjugate; PCV13 (Wyeth) which will in addition to PCV7 VTs contain serotypes 1, 3, 5, 6A, 7F and 19A. This vaccine has undergone phase I trials that have been published (285) and phase II and bridging studies are ongoing.

The limitation of PCV vaccines is the number of serotypes that can be contained in the vaccines. Therefore development of protein based vaccines that potentially may cover

the majority of pneumococcal serotypes may be the optimal solution (116-118). The coverage of different PCVs in different populations is listed in Table 4.

Table 4. Serotype coverage of different PCVs.

Vaccine/valences	Manufacturer	Serotypes	Geographical area	Coverage.	References
PCV7	Wyeth	4, 6B, 9V, 14, 18C, 19F, 23F	Iceland USA W-Europa Africa Asia	51% >80% 66-80%% 50->80% <50%	(286) (20, 95, 287)
PHiD-CV	GSK	1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F 23F	Iceland USA W-Europe Africa Asia	72% >80% 66->80% 66-80% 50-65%	
PCV13	Wyeth	1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F	Iceland USA W-Europe Africa Asia	82% >80% >80% 65->80%% 50-80%	

The table shows the corverage of the currently licensed PCV7 (Prevnar/Prevenar), the vaccine PHiD-CV (NAME, licenced in Canada) and experimental PCV13-CRM_{197}, their manufacturers, serotypes in the vaccines and the percentage coverage of pneumococcal serotypes in Iceland and four geographical areas in the world. Reviewed in (95).

It is clear that many questions still remain regarding use of the current PCVs and more questions will arise as their usage increases.

In this thesis we raise several questions regarding six formulations of PCVs, safety, immunogenicity, protective capacity assessed *in vitro* and *in vivo*, the effect of mixed carrier proteins, carrier-interference, generation of short term and long term memory and the effect of PPS booster, as well as the effect on nasopharyngel carriage and AOM.

AIMS

The main aim was to investigate safety and immunogenicity of pneumococcal conjugate vaccines in infants.

Safety. Are the pneumococcal protein conjugate vaccines safe when injected in infants? (Trial I, Paper I and II; Trial II, Paper III and IV; Trial III, Unpublished data; Trial IV, Unpublished data; Trial V. Paper V).

Immunogenicity

1. Does pneumococcal conjugate vaccine induce functional antibodies in infants? (Trial I, Paper I and II; Trial II, Paper III and IV; Trial III, Unpublished data; Trial IV, Unpublished data).
2. The search for optimal protein carrier for each serotype. Can the immune responses be enhanced to the poorly immunogenic serotypes? (Trial II, Paper III and IV; Trial III, Unpublished data; Trial IV, Unpublished data).
3. Do two carrier proteins for the poorly immunogenic serotypes enhance the antibody responses?
4. How many primary doses are needed in infancy, to generate sufficient antibody responses and immunological memory? (Trial V, Paper V).
5. Generation of immunological memory (Trial III, Unpublished data; Trial V, Paper V; Trial II, Unpublished data).

Effect of pneumococcal conjugate vaccines at mucosal level.

1. Nasopharyngeal colonization (Trial II and IV, Unpublished data).
2. Nasopharyngeal colonization and relationship with serotype specific IgG antibody responses (Trial IV, Unpublished data).
3. Effect of pneumococcal conjugate vaccination on otitis media and antibiotic usage (Amendment to Trial IV, Unpublished data).

MATERIAL AND METHODS

This thesis is based on five clinical trials on pneumococcal conjugate vaccines and additional studies to them. The primary research aim was to investigate safety and immunogenicity of six PCV formulations by conducting one phase I trial in toddlers and three phase II trials and one phase III trial.

Study population

In all the trials, parents of healthy Icelandic infants, toddlers or adults were offered to participate in a study on a new vaccine against the bacteria *Streptococcus pneumoniae*. The studies were all performed in conjunction with regular well child care visits as recommended by the Icelandic health authorities. In all the trials the parents received a letter of introduction which was followed up with either a clinical visit or phone call by their regular nurse taking care of the newborn at each health centre.

In the phase I trial (Trial III, unpublished data) parents of healthy toddlers were contacted when the children were 17 months of age and offered to participate in a safety study on 11-valent PCV. The children were vaccinated with one dose of a pneumococcal conjugate vaccine, PCV11-F3bis and followed up at time of Measles, Mumps and Rubella (MMR) vaccination at 18 months. Safety was observed at 30 minutes, 5 days and 28 days with a follow-up visit at day 7 and 28

The participants in the phase II trials were healthy full term infants born in Iceland and recruited and followed up during well child visits in trials I, II and V at the following health care centres; Centre for Child Health Services and Arbaer Health Centre in Reykjavik, Solvangur Health Centre in Hafnarfjordur and Borgir Health Centre in Kopavogur. In trial IV the mothers of newborn infants in the obstetrical ward at Landspitali University Hospital were invited to take part and then followed up at the Centre for Child Health Services in Reykjavik. Fifteen healthy adult volunteers (median age 28.9 years) were recruited in trial I (Paper II) and vaccinated with one dose of Pn6B-TT. Blood samples were obtained before and 4 weeks after vaccination.

During the first study visit, a study physician gave the participants detailed information and an informed consent form was signed.

Vaccines studied and study designs

The clinical trials and their designs are listed in Table 5, with the years of conduct, contents and producers of the trial PCVs administered as primary vaccination and type of booster vaccination. It shows also the study designs, number of recruited infants, age at vaccination and blood drawing, safety monitoring in days, outcomes and additional studies added to the trials.

The primary vaccinations were administered at the same time as regular infant immunizations according to the official Icelandic recommendations at the time of each study, namely at 3, 4 and 6 months for Trial I, II and IV (Paper I, II, IV and unpublished data) and at 3 and 5 months in Trial V (Paper V). However, in trial I the regular immunization schedule was compared to one with PCV vaccinations at 7 and 9 months in order to obtain information on age dependent immune responses to the vaccine (Paper I) and in trial V a schedule of two primary dose vaccinations at 3 and 5 months was compared with a schedule of three dose primary vaccinations at 3, 4 and 5 months (Paper V). Booster vaccination was administered in toddlers in conjunction with the regular toddler doses, at 18 months in trial I (MMR administered at follow-up at 19 months, Paper I and II), at 13 moths in trial II and IV (regular vaccines administered at follow-up at 14 months, Paper IV and unpublished data) and at 12 months in trial V (at same time as regular vaccines, Paper V) (Table 5).

Table 6 shows a detailed list of the trial vaccines and their contents, used in each clinical trials. The table also shows information for the concomitant regular vaccines and booster vaccines. The trial names are listed in footnotes. All vaccinations with the trial vaccines were administered intramuscularly in the thigh with concomitant vaccine injected in the contralateral thigh.

In Trial I, we investigated a mono-valent pneumococcal polysaccharide of serotype 6B conjugated with Tetanus Toxoid carrier protein (6BTT), provided by the National

Institutes of Health and Child Development, Bethesda, MD, USA (Lot 55683, FDA BB IND NO 1977, NIH Protocol NO. 88-2CH-163) (Paper I and II).

In Trial II, we compared two investigational octavalent pneumococcal conjugate vaccines containing capsular polysaccharides of serotypes 3, 4, 6B, 9V, 14, 18C, 19F and 23F conjugated with either diphtheria toxoid (PCV8-DT) or tetanus protein (PCV8-TT). The vaccines were produced by PASTEUR MERIEUX Sérums & Vaccins (later Aventis Pasteur and currently sanofi pasteur), Lyon, France (Paper IV). In that study half of the children (40 from each vaccine group) had nasopharyngeal cultures obtained at each visit and a group of 40 unvaccinated controls was recruited for nasopharyngeal culturing only. In addition to that study we offered those who received the PCV8-TT to participate in a study on immunological memory at 7 years of age, when we challenged them with a fractional dose of PPV23.

In the phase I Trial III and phase II Trial IV, we investigated two 11-valent pneumococcal conjugate vaccines (Trial III, formulation PCV11-F3bis and in Trial IV; formulation PCV11-F3 and PCV11-F3bis) provided by Aventis Pasteur, Lyon, France (now sanofi pasteur). In the PCV11-F3 formulation, PPS of serotypes 1, 4, 5, 7F, 9V, 19F and 23F were conjugated to tetanus protein and serotypes 3, 14, 18C and 6B conjugated to diphtheria toxoid. The PCV11-F3bis formulation contained the same formulations but both carrier proteins for serotypes 6B, 9V, 18C and 23F (Unpublished data). Amended to this trial was a follow-up at 18 and 24 months of age at which time nasopharyngeal culture swabs were obtained and at 24 month a blood sample for measurements of serotype specific IgG antibodies. At 24 months, a control group of playmates at same age was recruited for those who attended group child care at that time.

In Trial V, we investigated a mixed 9-valent pneumococcal polysaccharide and meningococcal C polysaccharide vaccine conjugated with the diphtheria toxoid mutant, CRM_{197} (9vPncMnCC), produced by Wyeth Vaccines Research, Pearl River, NY, USA. Each dose contained pneumococcal polysaccharides from serotype 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F and meningococcal group C oligosaccharide coupled to the mutated diphtheria protein, CRM_{197} (Paper V).

Table 5. List and design of trials supporting the PhD thesis and their trial design

Trial/Paper Phase	Time of conduct	Pneumococcal conjugate vaccine[1] *Producer* Trial code	Serotypes and protein carrier in PCV vaccine	Booster vaccine	Concomitant Vaccines (regular vaccination)	Design	Size N	Age (mo) Primary	Age (mo) Booster	Safety (days)	Blood samples Age (mo)	Outcome	Additions to the studies
I / Paper I-II Phase II	1993-1995	**6B-TT** *NICHD*	6B + Tetanus Toxoid	6BTT	DTwP and PRP-D at 3, 4, 6, 14 months IPV at 4, 6, 7 and 14 months	Open Safety Immunogenicity	21 19	3, 4 , 6 7, 9	18 18	Phone call at 24 & 48 hours	3, 4, 6, 7, 17, 18, 24, 30 7, 9, 10, 17, 18, 24, 30	Safety IgM, IgG, IgA, IgG1/IgG2 OPA	Saliva IgA NPC
II / Paper III-IV Phase II	1995-1997	**PCV8-TT** or **PCV8-DT** or **none** *Aventis Pasteur* **PNC150295**	3,4,6B, 9V, 14,18C, 19F, 23F + Tetanus protein or Diphtheria toxoid	PCV8-TT or PCV8-DT or PPV23	Tetravalent DTwP/PRP-T at 3, 4, 6, 14 months IPV at 4,6,7,14mo	Randomized Double blind (controls open) Safety Immunogenicity	160 PCV 40 controls	3,4,6	13	4 days, Diaries & Phone calls.	3,4,6,7,13,14	Safety IgG, IgG1/IgG2 Avidity, OPA	NPC at 3,4,6,7,10,14,18,30 Vaccinees and controls followed up at 7 years with fractional PPV challenge and IgG, IgG1/IgG2 measurements Passive mouse protection with infant sera
III / Unpublished Phase I	1997-1999	**PCV11-F3bis** *Aventis Pasteur* **PNF08297**	1,3,4,5,6B, 7F, 9V, 14,18C, 19F, 23F + Tetanus protein and/or Diphtheria toxoid. Both carrier proteins for serotype 6B, 9F, 18C and 23F)	PPV23	NA	Open Safety Immunogenicity	20	17	27	7 and 28 diary	17, 18, 27 and 27 + 1 week	Safety IgG, IgG1/IgG2 Avidity, OPA	Memory induction as demonstrated 10 months later by a PPV23 booster with measurements of IgG responses and avidity maturations
IV / Unpublished Phase II	1997-1999	**PCV11-F3 or PCV11-F3bis** *Aventis Pasteur* **PNF09297**	1,3,4,5,6B, 7F, 9V, 14,18C, 19F, 23F + Tetanus protein and/or Diphtheria toxoid (PCV11-F3bis contained both carrier proteins for serotype 6B, 9F, 18C and 23F)	PCV-F3 or PCV11-F3bis	Tetravalent DTwP/PRP-T at 3 mo, Pentavalent DTwP/PRP-T/IPV at 4, 6, 14 months IPV 7 mo	Randomized Double blind Safety Immunogenicity	146	3,4, 6	13	7 days diary	3, 6, 13, 14	Safety IgG, IgG1/IgG2 Avidity, OPA	NP at 4,7,10,14,18,24 Unvaccinated playmates at 2 years as controls for serum antibody measurements, NPC and history of otitis media.
V / Paper V Phase III	2000-2003	**9vPnCMnCC** Wyeth **D139-P506**	1,4,5,6B,9F, 14,18C, 19F,23F and Meningococcus C + CRM_{197}	9vPnCMnCC or PPV23 + MnCC	Pentavalent DTaP/ PRP-T/IPV at 3, 5 and 12 months	Randomized Safety Immunogenicity	224	3,5 or 3,4,5	12	7days	3,6,12,13	Safety IgG	Extra blood sample was obtained from 61 infant 7 days after booster vaccination to explore kinetic of memory response.

The table lists the trials performed, pneumococcal conjugate vaccines tested, booster vaccine and concomitant vaccines. It shows the trial design, number of participants and outcome measures. In the last column are listed additional studies in each trial.

[1]The vaccines and their contents are listed in Table 6. PCV: Pneumococcal conjugate vaccine, NICHD: National Institute of Child Health and Development; Mo: months; OPA: Opsonophagocytic Assay; NPC: Nasopharyngeal cultures; PPV23: 23-valent pneumococcal polysaccharide vaccine; DTwP: Diphtheria/Tetanus/whole cell Pertussis; IPV: injectable polio vaccine; DTaP: Diphtheria/Tetanus/acellular Pertussis, MnCC: Meningococcal C conjugate.

Table 6 List of the trial vaccines and teir contents

Name of vaccine formulation	Vaccine producer, Lot number and trial code	Valencies Phase	Pneumococcal serotypes and quantity of each saccharide pr dose	Carrier protein and quantity	Adjuvant	Trial Name is in footnote
6BTT	NICHD (Lot 55683)	Monovalent PCV[1]	6B: 12 µg	Tetanus toxoid, 37,5 µg	none	I[1]
PCV8-DT	Aventis Pasteur (sonofi pasteur) (Lot 940115) (PNC150195)	8-valent PCV	3, 4, 6B, 9V, 14, 18C, 19F and 23F: 3 µg pr serotype	Diptheria toxoid	None	II[2]
PCV8-TT	Aventis (sonofi pasteur) (Lot S3004) (PNC150195)	8-valent PCV	3, 4, 6B, 9V, 14, 18C, 19F and 23F: 1 µg pr serotype	Tetanus protein	None	II[2]
PCV11-F3	Aventis Pasteur (sanofi pasteur) Lot S3496 (infant) and S3580 (booster) (PNF09297)	11-valent PCV	1, 4, 5, 7F, 9V, 19F and 23F: 1µg pr serotype 3, 14, 18C: 3µg pr serotype 6B: 10µg	1, 4, 5, 7F, 9V, 19F and 23F: Tetanus protein 3, 6B, 14, 18C: Diphtheria toxoid	None	III[3,4] & IV[4]
PCV11-F3bis	Aventis Pasteur (sonofi Pasteur) Lot: S3452. (PNF08297) (PNF09297)	11-velent PCV	1, 4, 5, 6B, 7F, 9V, 18C, 19F, 23F: 1 µg pr serotype conjugated to Tetanus Protein 3 µg: 3, 6B, 9V, 14, 18C, 23F conjugated to Diphtheria Toxoid	1, 4, 5, 6B, 7F, 9V, 18C, 19F, 23F: Tetanus protein 3, 6B, 9V, 14, 18C, 23F: Diphtheria toxoid.	None	III[3,4] & IV[4]
9vPnCMnCC	Wyeth Lot: 006A_002B (infant doses), Lot: 009A_004A (infant and booster doses) (D139-P507)	9-valent PCV plus Meningococcus C conjugate.	1, 4, 5, 9V, 14, 18C, 19F and 23F: 2 µg pr serotype, 6B: 4 µg Meningococcal group C oligosaccharide: 10 µg	38.5 µg CRM_{197}.	Aluminium phosphate	V[5]
PPV23 (Pneumo23®)	Sanofi Pasteur MSD	23-valent pneumococcal polysaccharide vaccine:	1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F: 25 µg of each capsular polysaccharides			II[2] and V[5]

The table shows all the pneumococcal conjugate vaccine and 23-valent polysaccharid vaccine used in the trials that support this thesis. The vaccine producer are listed with Lot numer of the vaccine used, the valencies of each formulation, quantity of polysaccharides for each serotype and conjugate proteins and adjuvant in the vaccines. NICHD: National Institude of Child Health and Developement. PCV: pneumococcal conjugate vaccine.

1) "Immune responses of infants vaccinated with serotype 6B pneumococcal polysaccharide conjugated with tetanus toxoid"

2) "Study on the functional activity of antibody response elicited by two different octavalent vaccines of pneumococcal types 3, 4, 6B, 9V, 14, 18C, 19F and 23F conjugated with tetanus or diphtheria toxoid in infants",

3) "Assessment of the safety and immunogenicity of an eleven-valet mixed tetanus or diphtheria pneumococcal polysaccharide conjugate vaccine, when combining carriers for serotypes 6B, 9V, 18C and 23F in healthy Icelandic toddlers"

4) "Assessment of the safety and immunogenicity of two different formulations PCV11-F3 and PCV11-F3bis of a pneumococcal polysaccharide (Type 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) conjugate vaccine in healthy Icelandic infants"

5) "A phase III randomized, pulticentre study evaluating the safety and immunognicity of a 9-valent pneumococcal, meningococcal group C saccharide conjugate combination vaccine administered either at 3, 5 and 12 months of age or at 3, 4, 5 and 12 months of age in healthy infants"

Ethics

All the trials were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice and were approved by the appropriate ethics committee in Iceland but during the course of the trials different ethics committees were responsible. Trial I was approved by the Ethics committee of Landspitali University Hospital (07.06.93 and 06.05.93), Trial II and its amendments, by the ethics committees of Landspitali University Hospital in Reykjavik (4/95-96 and 30/95-96 and 45B/97-98) and of the Icelandic Medical Association, Trial III and IV by the Ethics Committee of the Directorate of Health in Iceland March 19, 1998; and Center for Biologics Evaluation and Research (CBER) April 20, 1998 and trial V by the National Bioethics Committee # 01097AG2. The trials were reported to the Personal Data Protections Agency, Reykjavik.

Outcome measures.

Safety.

The safety was monitored by diary recording and home phone calls by a study nurse. The parents recorded local reactions including erythema, induration, tenderness and swelling. Systemic reactions included fever monitored by daily rectal temperature measurements, decreased appetite, continuous crying, rash, decreased activity, drowsiness, insomnia, vomiting and diarrhoea. Side effects were then recorded in the study files by a study nurse and physician during next clinical visit. The parents were informed to call the study nurse if anything unexpected happened and in case of severe adverse reactions or hospitalization from any cause. In studies II – V, all severe adverse events were reported immediately to the vaccine producer with a follow-up report on the event (Paper I, IV and V and unpublished data).

In Trial I, 1993, parents kept diary at 6, 24 and 48 hours after vaccination and received a phone call from a study nurse on day three. In Trial II, parents kept diary for 3 days with a phone call on day 4 and return of the diary at next clinical visit. In Trial IV the parents

kept diary for 5 days, reviewed and recorded in the study files by the investigator during next visit and in Trial IV for diary was kept for 7 days plus 28 days recordable events after each vaccination.

In Trial II and IV on the PCV8-DT/PCV8-TT conjugates, the participants were followed for severe adverse events (SAE) from enrolment until 28 days after booster dose, at 14 months. In Trial V, on the 9vPnCMnCC conjugate all adverse events were monitored from study enrolment through to 28 days following the 5 month visit and from the 12 month visit and the following 28 days. From the 6 month visit to the 12 month visit all deaths and life-threatening events were reported as serious adverse events, within 24 hours of the investigator becoming aware of the event

The concomitant vaccines were different in all the trials, most significant for Trial I, II and IV in which the whole cell Pertussis component was used whereas the acellular Pertussis was used in Trial V. In Trial I, DTwP, Hib-PRP, and IPV were all administered with separate injections, in Trial II, DTwP and Hib/PRP-D were in one injection and IPV in another. In Trial IV the IPV was in same injection as DTwP –Hib/PRP-T when given concomitantly and in Trial V all concomitant vaccines were administered in one injection (DTaP-PRPT-IPV) at same time as the trial vaccine.

Immunogenicity and functional capacity

Immune responses to the study formulations were evaluated by serotype specific antibody measurements in blood samples obtained before and four weeks after primary and booster vaccinations. The functional capacity of the vaccine-induced antibodies was evaluated *in vitro* by an opsonophagocytosis assay (OPA) and *in vivo* in a mouse protection model using sera from vaccinated toddlers and infants. In Trial I and II blood sample was obtained at each visit (Paper I and II) except in Trial II where half of the infants had a blood test at 4 months and the other half at 6 months in order to evaluate the kinetics of the antibody responses (Paper IV). In Trial III (unpublished data) and V (Paper V) blood tests were done before and 4 weeks after primary series and booster dose. In Trial III and V and an additional study to Trial II at 7 years (Unpublished data), parents were offered to come for an extra visit one week after the booster injection for

evaluation of the immunological memory responses by measuring the kinetics of serotype specific IgG responses.

Measurements.

ELISA

All measurements except for Trial V (Paper V) were done at the Department of Immunology at Landspitali University Hospital in Reykjavik.

The ELISA for Trial I is descried in details in Paper I and II. In essence plates (Costar, Cambridge, MA) were coated with 10 µg/mL 6B polysaccharide (American Type Culture Collection (ATCC), Rockville, MD). All sera were pre-adsorbed with the cell wall polysaccharide (CWPS, Statens Seruminstitut, Copenhagen Denmark). 6B IgG was detected with biotin-labelled monoclonal antibody HP-6043 (Hybridoma Reagent Laboratory, Baltimore, MD) followed by incubation with alkaline phosphatase-labeled avidin (DAKO, Glostrup, Denmark). The reaction was developed by p-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO) and optical density was read at 405 nm in a Titertek Multiscan® spectrophotometer (Flow Laboratories, Irvine, Scotland). The IgM and IgA isotypes were measured with similar ELISA using the same type of plates, coated with 20 µg/mL of 6B polysaccharide (ATCC), CWPS adsorption and incubated with alkaline phosphatase-labeled conjugates, monoclonal antibody for IgM (1/500, clone MB-11, Sigma) or purified rabbit antibodies to human IgA (1/500, D338, DAKO). Laboratory standard prepared from pooled adult post-vaccination (Pneumo23, Pasteur Merieux, Lyon, France) sera was calibrated against a reference serum 89-SF, provided by Dr. Carl E. Frasch, Food and Drug Administration. Results were expressed in µg of antibody /ml calculated from the standard curve.

In Trial II, III and IV, (Paper IV and unpublished data) some modifications were made to the ELISA protocol used. The ELISA method was during this time undergoing development which has been published (141). The consensus ELISA is evolved from the methods originally described by Quataert et al (288), same method as used by Wyeth who performed the antibody measurements in Trial V (Paper V). In essence, the ELISA used in all our studies was according to the consensus protocol at the time of each study.

In trials II-IV MaxiSorp microtiter plates (MaxiSorp; Nunc AS, Roskilde) were used and coated with optimal concentrations of (2.5 to 10 μg/mL) of the pneumococcal polysaccharide (ATCC, Manassas, VA, except 6A from Aventis Pasteur) except in Trial II where 10 μg/mL were used for all serotypes. Plates were incubated at 37°C for 5 hours, followed by blocking with 10% foetal calf serum in PBS (F-PBS) for 1h at 37°C. Sera and standard (89-SF, from Dr. Carl Frasch, FDA, Bethesda, USA) diluted (I/50 in trial II, 1/100 in trial III and IV) in F-PBS containing 10 μg/mL CWPS (Statens Seruminstitut, Copenhagen, Denmark), and incubated for 30 min at RT. Serial dilutions of sera and standard (100 μl/well) incubated for 2h at 37°C. In Trial II, bound IgG was detected by 2 h of incubation with monoclonal antibody to human IgG, HP-6043-HRP (Hybridoma Reagent Laboratory, Baltimore, MD). The reaction was developed by tetramethylbenzidine (Kirkegaard & Perry Labs Inc., Gaithersburg, MD), and the reaction was stopped by addition of 0.18 M H2SO4. In Trial III and IV, goat anti-human IgG conjugated to alkaline phosphatase (Sigma) was used, diluted 1/3000 in F-PBS (100 μl/well) for 2h at 37°C. Incubation was with the substrate (100 μl/well) *p*-NPP (Sigma) in diethanolamine buffer (pH 9.6), for 1 h at 37°C.

Optical density was measured at 450 nm in an ELISA spectrophotometer (Titertek Multiscan Plus MK II; Flow Laboratories, Irvine, UK). Calculation of antibody concentration was by four parameter logit-log transformation, using the CDC-ELISA program developed by Brian D. Plykaitis (CDC, Atlanta, USA). Results were expressed in geometric mean concentrations (GMC) in μg/mL and rate of responders (≥ 0.15, 0.3 and 1.0 μg/ml). Assignment of anti-6A IgG level in the 89-SF was at the time not available and therefore 6A IgG levels were arbitrarily given the 6B IgG assigned levels but expressed in arbitrary units per ml (AU/ml).

ELISA for Trial V on 9vPnCMnCC was performed by Wyeth Vaccines Research at ARUP in Salt Lake City, Utah. USA as previously described in (139, 288). This was done by the request of the vaccine producer, Wyeth, in order to maintain consistency in antibody measurements between their vaccine trials. It differs from the currently recommended consensus ELISA protocol in the following ways; Nunc Microwell plates (catalog no. 2-69620) are used and coated with optimal coating concentrations for each serotype diluted in sterile PBS; 0.5 μg/mLfor Pneumococcal serotypes 4 and 9V; 1

µg/mLfor serotypes 7F, 14, and 18C; 2 µg/mLfor serotype 1; 5 µg/mLfor serotype 5; and 10 µg/mLfor serotypes 3, 6B, 19F, and 23F. In stead of using CWPS pre-adsorption, the sera was pre-adsorbed with a 1:50 dilution of 2.5 µg/mol Pn ELISA absorbent (lot B) containing soluble capsular components including CWPS prepared from a serotype-specific capsule-negative variant (CSR-II) of *S. pneumoniae.* The antibody concentration in the 89-S were quantified by a precipitation method (288). Anti-PPS antibodies to serotypes 6B, 14, and 18C were quantitated by precipitating 100 ml of the standard reference serum lot 89-S with 100 ml of each antigen separately. The PnPs concentrations ranged from 1.25 to 20 µg/mL in 0.01 M PBS. The mixture was incubated in glass tubes for 1 h at 37°C and then for 1 to 4 days at 4°C. The precipitate was centrifuged at 900 x g for 1 h at 4°C and washed twice with PBS. The precipitate was dissolved in 0.02 ml of 0.1 N NaOH. Antibody protein concentrations were then determined with a bicinchoninic acid protein determination kit (Pierce, Rockford, Ill.; catalog no. 23225X).

IgG1 and IgG2 antibodies to 6B polysaccharide were measured by the same ELISA protocol as for IgG in each study, except that the detection of biotinylated MAbs to IgG1 and IgG2 (HP-6069 and HP-6002, respectively; Hybridoma Reagent Laboratory) diluted 1:2000 in PBS-Tween and incubated for 2 h and then for 30 min with HRP-streptavidin (Research Diagnostics) diluted 1:10000 in PBS-Tween. The reaction was developed by TMB as described above, and antibody levels were calculated from the 89-SF standard by using the values assigned by Soininen et al. (289).

Importantly, all the ELISA assays were performed according to internationally recommended ELISA protocols with minor modifications, using the same reference standard 89SF and CWPS neutralization. In none of the studies was pre-adsorption done with serotype 22F.

Radioimmunoassay

Total anti-6B antibodies were measured by radio-immunoassay (RIA) as previously described (136); the results expressed in ng of antibody N (Ab N)/mL (conversion factor for ng of Ab N/mL to antibody concentration is 6.25).

Measurements of antibodies to concomitant vaccines in Trial II (Performed at Aventis

Pasteur).

Anti-Hib (PRP) antibodies in Trial II was measured using a Farr-type Radio Immunoassay (RIA) based on specifications established by C.E. Frasch (Centre for Biologics Evaluation and Research, CBER, NIH, Bethesda, US). Excess antigen ^{3}H detected in the precipitate, compared to a calibrated reference antiserum. The anti-PRP antibody titre was expressed as µg/mL using a reference serum with a titre of 70 µg/mL provided by CBER, Lot No. 1983.

Diphtheria antitoxin by neutralisation on Vero cells

Anti-diphtheria response was measured by the ability of the test sera to protect Vero cells from a diphtheria toxin challenge. Using sterile 96-well microtitre plates, 2-fold dilution series of test sera was challenged with diphtheria toxin and allowed to incubate for one hour. Vero cells were then added, the wells sealed with sterile mineral oil and incubated for 6 to 8 days. Results were reported as IU/mL by comparison to a calibrated reagent (WHO Lot DIPH-1-93) and determined by the highest serum dilution that allowed cell metabolism in the presence of the challenge dose of diphtheria toxin.

Anti-Tetanus antibodies by ELISA

Anti-Tetanus antibody titres were determined by an ELISA. Tetanus toxoid (Aventis Pasteur, US) was absorbed to plastic microtitre wells and incubated with Goat Anti-Human Ig-specific antibody conjugated to alkaline phosphatase. A subsequent reaction with alkaline phosphatase substrate was measured in spectrophotometer. The amount of antibodies was calculated by comparison to an international human reference (WHO Lot TE-3) with assigned units by a Parallel Line Analysis method and expressed in IU/mL.

Opsonophagocytic Assay (OPA).

Detailed description on OPA is provided in paper I. In essence, sera were kept in aliquots at -70°C until analysed to preserve the complement activity and were assayed using fresh PMN and ^{3}H -labelled pneumococci without adding external complement. OPA was tested for the following serotypes and strains 6A; (strain DS2215-94) , 6B (Icelandic strain), 9V , 18C, 19F, 23F, using reference pneumococcal strains (ATCC)

except for 6B which was a clinical isolate from Iceland. Bacterial and PMN suspensions (150 µl of each, ratio of approximately 10:1) were mixed with test sera at a concentration (15% for infants, 5% for adults) predetermined to be in the sensitivity range of the assay. The total volume of 0.5 ml was incubated with rotation (250 rpm) for 30 min at 37°C. Controls for non-specific binding (NSC) (with all reactants except heat-inactivated FCS instead of human serum) and total bacteria input (TB) (with all reactants) were included in each assay. The reaction was stopped by adding 2 ml of phosphate-buffered saline-0.02% NaN3. The PMN and the cell-associated bacteria (CAB) were pelleted by centrifugation at 160 x *g*, except that TB was centrifuged at 2,200 x *g*. After washing, cell pellets were resuspended in 0.5 ml of 1.25% deoxycholate and transferred to 4.5 ml of scintillation liquid (Hionicfluor; Packard, Greve, Denmark). The radioactivity (range, 500 to 10,000 cpm) was measured in a liquid scintillation counter (Packard) and percent uptake of ^{3}H -labelled bacteria was calculated as (counts per minute of CAB - counts per minute of NSC)/(counts per minute of TB - counts per minute of NSC) x 100. Opsonic activity (OA) was expressed in AU calculated from a standard curve of an adult post vaccination serum pool that was assigned 100 AU. The lower detection limit was 2,5-5 AU depending on the serotype and strain used (151).

Avidity.

Avidity of IgG antibodies was measured by ELISA. The protocol was the same as for IgG, including incubation with serial dilutions of KSCN (7,5 - 0,12 M) for displacement of bound antibodies by the chaotropic ion SCN-, which disrupts the non-covalent antigen-antibody binding in a dose dependent manner related to the affinity of the antibody to its epitope. Results are expressed as avidity index, AI. One AI = M KSCN needed to displace 50% of antibodies (Paper III).

Nasopharyngeal culturing

Nasopharyngeal swabs, were collected with Mini-tip Culturette®, (Becton Dickinson and Company, Cockeysville, MD) and cultured selectively for pneumococci within 8 hours using blood agar plates with and without gentamicin (5 mg/l). The pneumococci

were serotyped by coagglutination 32 with antisera from Statens Seruminstitut, Copenhagen, Denmark.

Passive protection in mice.

The mouse protection model was used to test randomly selected serum from 59, fourteen month old infants who were vaccinated with the 8-valent PCV8-TT vaccine at 3, 4 and 6 months and with same conjugate or the PPV23 vaccine at 13 months, against serotype 6B and 6A blood and lung infection. The model has been described elsewhere (290).

In essence, outbred 8-week-old female NMRI mice (Bomholtsgard, Denmark) were injected intraperitoneally with 59 randomly selected infant serum samples into 2 mice each (175 μL of undiluted serum per mouse) 3 h before intranasal challenge with pneumococci: Twentynine serum samples obtained from infants primed and boosted with PCV8-TT and 30 serum samples obtained from infants primed with PCV8-TT and boosted with PPV23. Two sets of experiments were performed in which the mice were challenged with either serotype 6A or 6B, using the same serum samples for passive immunization. The inoculum dose of serotype 6B was 10^7cfu/mouse, and mice were bled at 12, 18, and 24 h. Detection limit of 6B bacteremia was 2.26 log cfu/mL in blood. The inoculum dose of serotype 6A was $53x10^6$ cfu/mouse, and mice were bled at 18 and 24 h. Detection limit of 6A bacteremia was 3 log cfu/mL in blood. Mice with bacteremia below the detection limit were considered to be protected. 6B-challenged mice with detectable bacteremia were considered to be not protected. Mice challenged with 6A were considered to be not protected if they had bacteremia >5 log cfu/mL; but considered to have reduced bacteremia 3–5 log cfu/mL.

The mice were anesthetized and challenged intranasally with pneumococci in 50 μL of saline. To evaluate bacteremia, blood samples obtained from the tail vein at various times after challenge were diluted in sterile saline, and were plated on blood agar for live counting of pneumococci (expressed as colony-forming units per milliliter of blood). After 24 h, the mice were killed, and the lungs were removed and homogenized, and then diluted to 3 mL of sterile PBS. Serial dilutions were plated on blood agar (Difco Laboratories, Detroit, MA, USA) with selective supplement for staphylococci and

streptococci containing nalidixic acid and colistin sulphate ((SR0070), Oxoid Ltd. Wade Road, Basingstoke, Hants, UK), to determine colony forming units per lung.

Statistical analysis

In Trial I all comparisons on antibody levels were performed on log transformed data. Using Sigma Stat software (Jendel Scientific, Erkrath, Germany, Version 2.01), a paired t-test was used to compare responses within a group and unpaired t-test between groups. Wilcoxon Signed Rank Test was used when normality test failed.

In Trial II the data was analysed with SAS software (SAS Institute, NC, USA, Windows 6.08). All calculations were done on log transformed data and individual pre and post vaccination sera compared with paired t-test for each serotype and unpaired between groups. The opsonic activity after primary and booster vaccinations were compared to opsonic activity of pre-vaccination pool by Mac Nemar test for $\geq$ 10, 25 and 50 AU.

In Trial III and IV the results were similarly analysed with the SAS software and based on log transformed data using t-tests. The difference between the PCV11-F3 and PCV11-F3bis was tested by constructing the 95% CI of the difference in seroresponse rate.

In Trial V the statistical calculations were done using SAS software. For the antibody concentrations and titres, geometric means and associated 95% CI were based on mean and 95% confidential interval of log transformed data which was then transformed to original scale. The ratio of geometric means between the two treatment groups and the corresponding 90% CI was based on the mean of log transformed ratios. The two-sample t-test statistic of the mean difference was used to form the 90% CI. The estimated difference and the CI was then exponentiated to obtain an estimate of geometric mean ratio and its 90% CI. For proportions within each treatment group, exact 95% CI was calculated.

In the additional studies performed to trials II, III and IV, the calculations were performed with GraphPad Software Inc. (www.graphpad.com), using log transformed data with a paired t-test to compare responses within a group and unpaired t-test between

groups. Nonparametric test, Wilcoxon Mann-Whitney, was used when normality test failed. Similarly, the ratios of IgG1/IgG2 were calculated for each child and log transformed for comparison with ANOVA when passing normality test but with nonparametric test using Wilcoxon-Mann-Whitney test when the data were not normally distributed.

Rate of side effects, nasopharyngeal colonization, otitis media and antibiotic usage were compared with Fisher's exact test using GraphPad Software Inc. (www.graphpad.com).

Correlation between antibody levels and OPA or AI was done with Pearson's correlation coefficient and protective efficacy by $\chi 2$ analysis.

Difference was considered significant if $p<0.05$.

RESULTS

Safety and immunogenicity of pneumococcal conjugate vaccines in Icelandic infants.

Safety.

Papers I, II, III, IV, V and unpublished data from Trial IV[2].

In the phase I trial, Trial III, the safety of 11-valent PCV11-F3bis formulation was investigated in toddlers. Twenty healthy toddlers at 17 months of age were immunized with a dose of PCV11-F3bis of who 17 received booster vaccinations with PPV23 vaccine at 27 months. Two mild local reactions with redness and swelling were recorded within 30 minutes. Twelve participants (60%) presented ≥ 1 local reaction (redness, skin warmth, swelling and indurations) which were usually mild on vaccination day and lasted ≤ 1 day, but one subject had severe pain with crying upon movement of the arm. Fourteen subjects (70%) presented with ≥ 1 systemic adverse event (fever, crying, irritability, anorexia and insomnia) on day 0 or 1 which lasted for no more than 4 days. Five children (25%) had fever ≥ 38°C. In the toddlers, all side effects were considered mild except in 3 children with 4 events. The four events were anorexia (two), irritability (one) and drowsiness (one) (Unpublished data).

In the three phase II and one phase III trial, a total of 570 infants were injected with 2117 doses of pneumococcal vaccine. Thereof, 1573 primary and 353 booster doses of PCV were administered and 191 PPV23 booster doses. Only 19 children (receiving 38

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[2] Abstract: S Sigurdardottir, Þ Gudnason, KG Kristinsson, S Kjartansson, K Davídsdóttir, G Ingólfsdóttir, M Yaich, O Leroy, I Jónsdóttir. Safety And Immunogenicity Of Two Different Formulations Of 11-Valent Pneumococcal Polysaccharide Conjugate Vaccines, F3 And F3bis In Healthy Icelandic Infants. The 2nd ISPPD, Sun City, S-Africa, 19-23 March 2000 (Appendix 2)

doses) of these did not receive concomitant vaccine with the primary vaccinations, namely the group B in the first study when vaccinated with the monovalent 6B conjugate at 7 and 9 months of age.

In Trial I**,** Local reactions were mild and infrequent. Temperature elevation to >38°C was recorded in 9%, of whom three had fever >38,7°C (Paper I, table 1).

For the other three trials, in which parents kept a formal diary, the total local and systemic side effects are summarized in Table 7 (Table 1 and 2 in Paper IV, and unpublished data).

In Trial II, on 8-valent PCV8-DT or PCV8-TT conjugates, the vaccines were equally safe during primary immunizations at 3, 4 and 6 months, and the local reactions were significantly fewer than those caused by the concomitant vaccines, DTwP//PRP-D ($P <$ 0.0001). After the booster vaccination at 13 months the PCV8-DT caused less local reactions compared to both PCV8-TT and PPV23, but side effects were comparable between the PCV8-TT vaccine and PPV23. During the infant vaccinations, the rate of fever $\geq$ 38°C caused either by the trial vaccine or the concomitant vaccines, was highest after the 1st vaccination, 58% and 68% for PCV8-DT and PCV8-TT, respectively and third vaccination, 69% and 79%, respectively, while after the 2nd vaccination the rate was 31% and 39%, respectively, as shown in Table 7 and in Paper IV, table 2. The booster dose which was administered at 13 months without a concomitant vaccine, showed a higher rate of fever after PPV23 than after conjuage booster; 44% and 50% for PCV8-DT-PPV23 or PCV8-TT-PPV23 groups, respectively but 18% and 29% for the PCV8-DT and PCV8-TT booster in respective group.

In Trial IV, on two 11-valent TT/DT conjugates administered concomitantly with DTwP/PRP-T or DTwP/PRP-T/IPV, systemic reactions (fever, drowsiness, irritability, crying, vomiting, diarrhoea, anorexia and insomnia) were observed for both PCV11-F3 and PCV11-F3bis, in 89% and 88% of subjects after the 1st dose, 66% and 64% after the 2nd dose and 84% and 85% after the 3rd dose, respectively. Thereof, fever > 38°C accounted for 32% and 33%, 19% and 30% and 60% and 68%, at each time point for each formulation, respectively. For both formulations local reactions were significantly fewer than caused by the concomitant vaccines containing DTwP/PRP-T/IPV (Table 7).

The PCV11-F3 formulation caused less total local side effects than PCV11-F3bis after the 3rd vaccination and collectively during the primary vaccination series, but there was not a significant difference. Most of the local reactions were mild or moderate. The number of severe reactions was greater in the PCV11-F3bis group with 5, 1, 2 and 8 severe local reactions after doses 1, 2, 3 and booster, respectively, compared with 0, 0, 2 and 2 in the PCV11-F3 group. In both the PCV11-F3 and PCV11-F3bis vaccine groups, higher number of infants showed at least one local side effect after the 3rd vaccination compared with 1st and 2nd vaccination. However, the local side effects were significantly more frequent after the concomitant vaccine containing DTwP/PRP-T ($P<0.0001$ for both vaccines at all time points) (Table 7).

In Trial V, primary doses of 9vPnCMnCC were given at 3 and 5 or at 3, 4 and 5 months concomitantly with DTaP/PRP-T/IPV. No difference was observed in safety whether administered in 2 or 3 primary doses. As summarized in Table 7, the trial vaccine caused any local reaction after the 3 month vaccination in 41% and 33% in 2- vs. 3-doses groups, respectively, in 40% after 4 months vaccination in the 3-dose group and in 38% and 33% after the 5 months dose, respectively. In this trial the local reactions were only compared between subjects receiving the same PCV in the two schedule groups but reactions from the concomitant vaccine were not recorded. Although not significant, the PPV23 booster caused higher rate of local side effects than the conjugate vaccine booster at 12 months of age. In the 2-dose group a local reaction was reported in 34% (95% CI: 20.1; 50.6) and 53.1% (95% CI: 38.3, 67.5) after 9vPnCMnCC and PPV23, respectively, and in 34.1% (95% CI 20.5; 49.9) and 51.1% (95% CI: 35.8, 66.3), respectively in the 3-dose group. Fever >38°C was recorded from 15% to 26% of subjects after the each infant vaccination but in 55% to 67% after booster doses with either the 9vPnCMnCC or PPV23. No differences were noted in rate of side effects after any of the vaccinations, whether the vaccine was administered in two or three primary doses.

Table 7. Percentage of infants with local, systemic and febrile adverse events.

	Clinical trial II Diary for 3 days				Clinical trial IV Diary for 5 days		Clinical trial V Diary for 7 days			
Trial vaccine (PCV) Concomitant vaccine CV)	**PCV8-DT[a] DTwP/PRPD**		**PCV8-TT[a] DTwP//PRPD**		**PCV11-F3 DTwP//PRPT**	**PCV11-F3bis DTwP//PRPT**	**2 doses 9vPnCMnCC DTaP//PRPT//IPV**		**3 doses 9vPnCMnCC DTaP//PRPT//IPV**	
	3 months vaccination				**3 months vaccination**		**3 months vaccination**			
Any local reaction[b]-PCV[f]	13		20		22	22	41		33	
Any local reaction- CV[f]	72		66		82	84				
Any systemic reaction[c]	95		96		89	88	86		77	
>38°C	58		68		32	33	24		22	
	4 months vaccination				**4 months vaccination**		**4 months vaccination**			
Any local reaction-PCV[f]	14		14		14	22	na		40	
Any local reaction- CV[f]	54		63		85	81				
Any systemic reaction	71		74		66	64	na		66	
>38°C	31		39		19	30	na		15	
	6 months vaccination				**6 months vaccination**		**5 months vaccination**			
Any local reaction- PCV[f]	28		29		37	45	38		33	
Any local reaction- CV[f]	70		82		88	84				
Any systemic reaction	89		94		84	85	78		67	
>38°C	69		79		60	68	26		25	
	Booster at 13 months				**Booster at 13 months**		**Booster at 12 months**			
	PCV8-DT	**PPV**	**PCV8-TT**	**PPV**	**PCV11-F3**	**PCV11-F3bis**	**9vPnCMnC**	**PPV + MnCC[d]**	**9vPnCMnCC**	**PPV+MnCC[d]**
Any local reaction[b]	13 [e]	69	38	55	43	41	34	53	34	51
Any systemic reaction[c]	51	64	59	75	53	51	84	92	76	80
>38°C	18	44	29	50	30	28	57	57	55	67

Paper 4 from Trial IV, unpublished data from Trial IV, Paper 5 and unpublished data from Trial V. The table shows percentage of children with any local, any systemic and febrile adverse effects after each vaccination in each vaccine trial comparing formulations or vaccine schedules. Rate of local reactions to concomitant vaccines is provided below the trial vaccine (Safety for concomitant vaccines not documented in trial IV).

PCV: Pneumococcal conjugate vaccine, CV: Concomitant vaccine.

[a] First and second doses were given concomitantly with DTwP//PRP-D; third dose was given concomitantly with DTwP//PRP-D and IPV.

[b] Any local reaction included erythema, induration and tenderness for trial IV and also swelling in trial II and III

[c] Any systemic reactions included fever, decreased appetite, continuous crying, rash, decreased activity and drowsiness and in trial II and III also vomiting and diarrhea and in trial III insomnia as well

[d] MnCC and PPV23 administered 2,5 – 5 cm apart in the left thigh.

[e] PCV8-DT vs. PPV: $p<0.0001$ and PCV8-DT vs. PCV8-TT: $p=0,0153$.

[f] PCV vs. CV in trial II and IV: $p<0.0001$.

Serious adverse events (SAE). In Trial II no SAEs were considered related to the trial vaccines. In Trial IV one out of 10 SAE was recorded as possibly related to the vaccine. An 8 month old girl was found to have neutropenia, 2 months after third infant dose of PCV11-F3bis which resolved 21 months later. Out of the other SAEs, not considered related to the trial vaccine, three occurred during primary vaccination period and consisted of gastroenteritis, insomnia plus otitis media and failure to thrive leading to hospital admission. The other six events occurring during the time period between age 6 and 12 months, were gastroenteritis and febrile seizure, RSV, urinary tract infection, epileptic seizure, haematoma due to fall and elective surgery for cleft palate.

In Trial V, three infants developed SAE leading to hospitalizations during the vaccination period and 28 days following last primary dose and 28 days after booster dose. Out of those, one developed asthma 29 days after 3rd vaccination, not considered related to vaccination. One developed septicaemia due to the non-vaccine pneumococcal serogroup 7, seven days after the 2nd vaccination, considered probably not related to vaccination. The same infant was 3 weeks later admitted for fever, otitis media and asthma and found to have neutropenia that later resolved. The third infant was admitted for viral gastroenteritis and dehydration 22 days after the booster dose, considered not related to vaccination.

In none of the trials was there a report of death or an adverse events leading to a discontinuation from the study.

Taken together, all six pneumococcal conjugate vaccines investigated in the five clinical trials, proved safe when administered to infants in two or three doses with a toddler booster dose with either the same conjugate vaccine or the PPV23.

Immunogenicity.

Paper I and II from Trial I, Paper III and IV from Trial II, Unpublished data from Trial III and IV and Paper V from Trial V.

In five clinical trials we investigated the immunogenicity of six PCVs, containing one to eleven pneumococcal serotype polysaccharides conjugated to DT, TT or CRM_{197} carrier proteins.

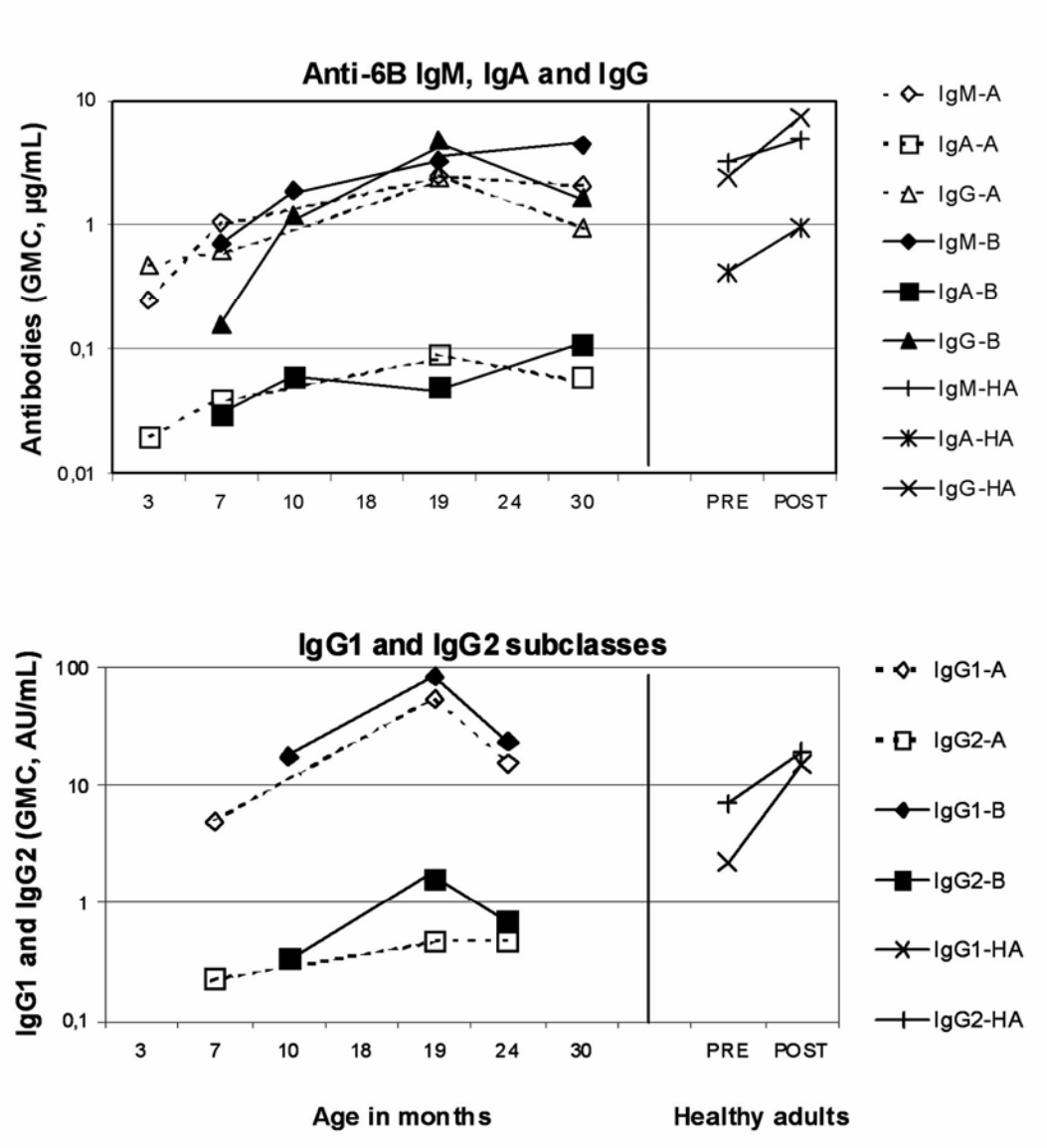
Does pneumococcal conjugate vaccine induce functional antibodies in infants?

Paper I and II, Trial I.

Antibody responses.

In Trial I we compared the antibody response elicited by a monovalent pneumococcal type 6B polysaccharide tetanus toxoid conjugate (6B-TT) in two different age groups, starting at 3 (Group A) or 7 (Group B) months of age. After initial decrease in 6B specific IgG, at 4 and 6 months, the 6B specific IgG response was significant after the third vaccination and increased to a geometric mean of 0.62 μg/mL($p = 0.005$, compared to pre-vaccination titres) in Group A at 7 months (Figure 2, Paper I). For group B, the post GMC 6B-IgG was 1.22 μg/mL at 10 months ($p < 0.001$, compared to pre-vaccination level). The post priming 6B specific IgG level in group A was significantly higher than in unvaccinated children at same age, at a GMC of 0.16 μg/ml (Group B, before the first injection) ($p < 0.01$). Total post-priming 6B specific antibodies measured by radioimmunoassay were 44 ng of antibody N/ml ($p < 0.053$) in Group A and 211 ng of antibody N/ml ($p < 0.001$) in Group B. Smaller increases in 6B specific IgM ($p < 0.001$) were observed and 6B specific IgA was detected post priming in both groups. Booster injection at 18 months elicited booster responses in total 6B and IgG antibodies; 62% of the infants in Group A and 79% of those in Group B had > 300 ng of antibody N/ml which at that time was considered a level of protection (Figure 1, Paper I).

Figure 2. 6B-TT induced specific IgM, IgA, IgG, IgG1 and IgG2 anti 6B.



The figure shows the geometric mean concentration in µg/mL for IgM, IgA and IgG anti- 6B but GMC in arbitrary units (AU)/mL for the IgG subclasses. The infants (left panels), vaccinated at 3, 4, 6 and 18 months (group A, open symbols) or 7, 9 and 18 months (group B, closed symbols) and healthy adults (HA, crosses) (right panels).

Furthermore, IgG subclasses were evaluated after primary vaccinations, after booster and at 24 month follow up visit in the infants and before and 4 weeks after the one dose

vaccination in healthy adults. Both IgG1 and IgG2 increased in the healthy adults. Infants reached adult levels of IgG1 anti-6B after the primary injections, although total IgG anti-6B was lower in both groups. After the booster injection the infant groups had total IgG- and IgM-anti-6B antibody levels similar to those of adults and IgG1 was the dominant infant anti-6B isotype at a level higher than in vaccinated adults. IgA and IgG2 antibodies remained at very low levels (Paper II, figure 1 and table 1).

Protective capacities.

In this trial the opsonic activities of post immunization sera were compared between the infant groups and healthy adults immunized once with the same vaccine.

Opsonic activity increased significantly after the 6B-TT vaccinations and correlated with 6B-specific antibody titres after initial and booster vaccinations in the infant groups. The highest opsonic activities among the infants were comparable to that of vaccinated adults. The opsonic activity correlated both with total 6B antibody levels in Group A ($r = 0.758$, $P < 0.001$) and in Group B ($r = 0.741$, $P < 0.001$) and with IgG anti-6B in Group A ($r = 0.741$, $P < 0.001$) and in Group B ($r = 0.653$, $P < 0.001$). Opsonic activity was highest in the sera with high levels of all 6B antibody isotypes (Paper I, figure 2, and Paper II, figure 3 and table 2). Thus, in this trial we demonstrated the functional capacity of the 6BTT-induced specific antibodies to opsonize the pneumococcus bacteria *in vitro.*

A serotype 6B PPS can be made immunogenic in infants when conjugated with a tetanus protein carrier. 6BTT elicited opsonic antibodies and memory responses in infants indicating a protective potential of the 6BTT vaccine. This also demonstrates the effect of age on the primary immune response, as the older group responded with higher specific antibodies, which may in fact also have been due to carrier priming since the older group (B) had previously been vaccinated with Tetanus toxoid as part of routine infant vaccination at 3, 4 and 6 months of age.

The search for the optimal protein carrier for each pneumococcal serotype. Can the immune responses to the poorly immunogenic serotypes be enhanced?

Paper III and IV from Trial II and unpublished data from Trial II, III and IV.

The next step was to search for the best protein carrier to elicit in infants the best antibody response to each serotype. Previously a 4-valent pneumococcal polysaccharide tetanus protein conjugate had been investigated in Finland, assessing immunogenicity and dose response (184, 224) to pneumococci conjugated to diphtheria toxoid or tetanus protein carriers. The results from those studies resulted in two pneumococcal conjugate formulations to be compared, one containing 3 μg of each serotype conjugated to Diphtheria toxoid (PCV8-DT) and the other 1 μg of each serotype, conjugated to tetanus protein (PCV8-TT). Our next trial compared these two formulations for eight serotypes followed by an 11-valent mixed carrier conjugate vaccine.

Comparison of Diphtheria toxoid and Tetanus protein as carriers in an 8-valent pneumococcal conjugate vaccine.

Antibody responses

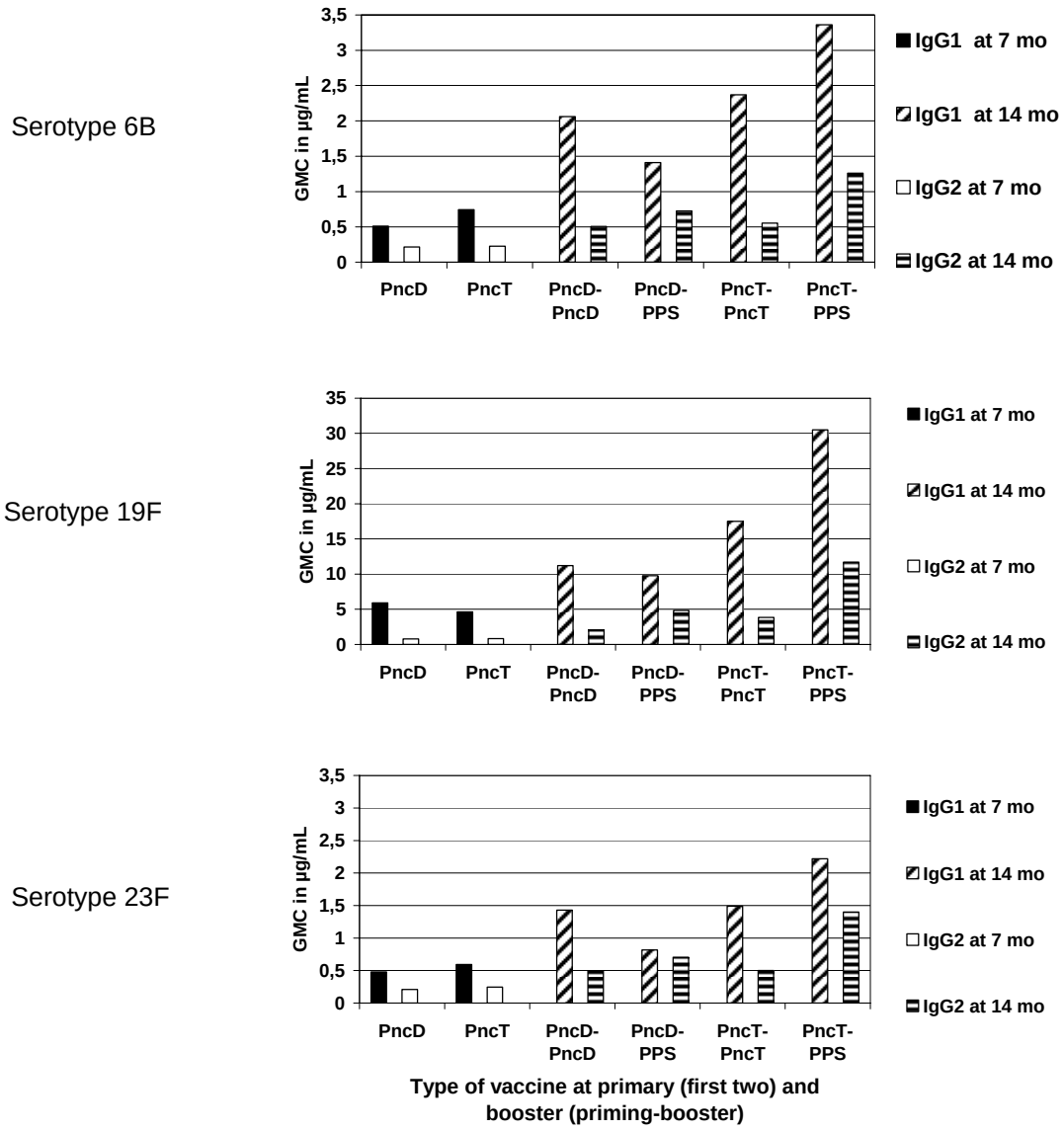
The immunogenicity of two octavalent pneumococcal conjugate vaccines including serotypes 3, 4, 6B, 9V, 14, 18C, 19F and 23F, conjugated either to tetanus protein (PCV8-TT) or diphtheria toxoid (PCV8-DT) were compared (See Table 5 for trial design). At 7 months, after priming, both groups had significant IgG response to all serotypes (Paper IV, Table 3, and Figure 1). The geometric mean concentration range was 0.35 to 4.09 and 0.65 to 3.38 μg/mL for PCV8-DT and PCV8-TT, respectively, with 88.2 to 100% and 92.4 to 100% of subjects reaching $\geq$ 0.15 μg/mL. The PCV8-DT induced higher primary responses to serotypes 3, 9V and 18C, whereas PCV8-TT gave higher response to serotype 4. The two conjugates elicited similar responses to the four remaining serotypes, 6B, 14, 19F and 23F. Good booster IgG responses to either

conjugate or the PPV23 vaccine were obtained in all vaccine groups; 97.5 to 100% of subjects reached ≥ 1 μg/mL. Overall, the highest booster responses were obtained in the group that received the PCV8-TT pneumococcal conjugate vaccine during primary vaccinations and booster dose of the PPV23 vaccine (Paper IV, table 3).

The IgG1 and IgG2 subclasses were measured for three serotypes, serotype 6B, 19F and 23F. The subclass pattern was in line with what was observed in response to the monovalent 6BTT. As shown in Figure 3 for the three serotypes, the IgG1 is the dominating antibody after both primary vaccination and booster with either the same conjugate or 23-valent polysaccharide vaccine. When the ratio of IgG1/IgG2 is calculated it was consistently lower in the groups receiving the PPV23 booster, both in the PCV8-DT and PCV8-TT group. No differences were observed between IgG1 and IgG2 antibodies after primary vaccinations in the PCV8-DT or PCV8-TT groups. The ratio IgG1/IgG2 was also comparable between the vaccine groups.

After the booster vaccination the ratio of IgG1/IgG2 anti-6B antibodies was significantly higher after the conjugate vaccine than after the PPV23 booster, which tended to induce higher IgG2 responses; IgG1/IgG2 for PCV8-DT vs. PPV23 booster was 5.11 vs. 1,94, respectively (p=0.0001) and for PCV8-TT vs. PPV23 booster 4.29 vs. 2.67, respectively (p=0.021). Priming with PCV8-TT and boosting with PPV23 gave highest levels of both IgG1 and IgG2 at 14 months but the differences between PCV8-DT and PCV8-TT groups at that age were only significant for IgG1 in the group who received the PPV23 booster (p=0.004). Similar patterns as for serotype 6B were observed in IgG1 and IgG2 responses to serotype 19F and 23F. For serotype 19F the IgG1/IgG2 ratio was 5.44 vs. 2.03 in response to PCV8-DT vs. PPV23, respectively in the PCV8-DT group (p=0.0001) and 4.53 vs. 2.61 for PCV8-TT vs. PPV23 booster in the PCV8-TT group (p=0.0034). The ratio of IgG1/IgG2 in response to serotype 23F was also comparable between the two conjugates but PPV23 booster induced higher IgG2 responses resulting in IgG1/IgG2 of 2.89 vs. 1.16 for PCV8-DT vs. PPV23, respectively in the PCV8-DT group (p=0.0001) and 2.99 vs. 1.59 for PCV8-TT vs. PPV23 booster in the PCV8-TT group (p=0.0006).

Figure 3. IgG1 and IgG2 subclass responses to PCV8-DT or PCV8-TT.



The figure shows serotype 6B, 19F and 23F specific IgG1 and IgG2 responses at 7 months after infant vaccinations with PCV8-TT or PCV8-DT at 3, 4 and 6 months and at 14 months after a booster vaccination with same conjugate or 23-valent polysaccharide vaccine at 13 months.

Interference (Trial II, Unbublsihed data[3])

To investigate possible interference between the PCV8-DT conjugate and the concomitant vaccine *Haemophilus influenzae* type b (Hib) polyribosylphoshat (PRP) conjugated to DT in this study we measured IgG antibody levels and rate of responders to PRP, TT and DT. The antibody responses to DT and TT were comparable in both vaccine groups whereas antibody levels to PRP were significantly lower in the PncD group compared to the PncT group. The rate of responders with anti-PRP > 0.15 µg/mL was also significantly reduced as shown in Table 8.

Table 8. Antibody responses to concomitant vaccines given with PCV8-DT and PNC8-TT

	Geometric mean concentration			**Rate of responders**					
	µg/mL			% > 0.15 µg/mL			% > 1.0 µg/mL		
Groups	PCV8-DT	PCV8-TT	p	PCV8-DT	PCV8-TT	p	PCV8-DT	PCV8-TT	p
PRP	0.18	0.34	0.0079	53	76	0.0026	13	23	0.139
	Geometric mean titers			**Rate of responders**					
	IU/mL			**% > 0.01 IU/mL**			**% > 0.1 IU/mL**		
	PCV8-DT	PCV8-TT	p	PCV8-DT	PCV8-TT	p	PCV8-DT	PCV8-TT	p
TT	2.15	1.86	0.245	100	100	Ns	100	100	ns
DT	1.91	1.98	0.79	100	100	Ns	100	100	ns

The table shows the antibody responses to PRP (µg/mL), TT (IU/mL) and DT (IU/mL) on the left panel and rate of responders on the right, at 7 months, one month after primary vaccinations with PCV8-D or PCV8-T at 3, 4 and 6 months concomitantly with DTwP/PRP-DT, IPV given at 6 months.

These results thus demonstrate interference between the PCV and Hib vaccines sharing the carrier protein DT in infants.

[3] Abstract: I Jonsdottir, S. Sigurdardottir, Th Gudnason, S Kjartansson, K Davidsdottir, KG Kristinsson, G Ingolfsdottir and O Leroy. Concomitant administration of octavalent pneumococcal polysaccharide conjugate vaccine, PNC-D and *Haemophilus Influenzae* conjugate vaccine, PRP-D, sharing the carrier DT, may induce interference in infants. The 2nd ISPPD, Sun City, S-Africa, 19-23 March 2000.

Protective capacities.

Opsonophagocytosis

Quality and functional activities of antibodies were evaluated *in vitro* by avidity measurements and opsonophagocytic assay (OPA) and further, the protective efficacy of the sera was investigated in an *in vivo* mouse model.

The OPA was performed for serotype 6B, 19F and 23F (Table 9, Unpublished data[4,5]).

Table 9. Opsonophagocytosis at 7 and 14 months, after priming with either PCV8-DT or PCV8-TT and booster with either same PCV or PPV23 vaccine

Serotype	Vaccine	At 7 months, post primary		At 14 months, post-booster			
		PCV8-DT N=41	PCV8-TT N=38	PCV8-DT-PCV8-DT N=39	PCV8-DT-PPV23 N=38	PCV8-TT-PCV8-TT N=33	PCV8-TT-PPV23 N=40
6B	**GMT (95% CI)**	27.8 [a] (21.9;35.4)	48.1 [a,] (39.8;58.0)	58.1 (45.7;73.8)	44.8 (33.7;59.5)	60.2 (45.6;79.3)	76.5 (60.3;97.0)
19F	**GMT (95% CI)**	35.3 (28.6;43.5)	32.5 (25.5;41.3)	23.2 (15.8;34.0)	27.9 [b] (17.9;43.5)	47.6 (31.3;72.3)	78.6 [b] (55.9;110)
23F	**GMT (95% CI)**	22.9 (17.8;29.5)	24.3 (18.8;31.5)	35.8 (27.0;47.6)	26.0 [c] (18.8;35.8)	32.0 (21.5;47.5)	54.6 [c] (40.9;72.8)

Unpublished data from Trial II. The table shows the opsonophagocytosis at 7 months, after primary vaccination with either 8-valent PCV8-TT or PCV8-DT and at 14 months, one month after booster vaccination with either same PCV or PPV23 vaccine.

a) PCV8-DT vs. PCV8-TT at 7 months: p=0,0005
b) PCV8-DT-PPV vs. PCV8-TT-PPV: p=0,0003
c) PCV8-DT-PPV vs PCV8-TT-PPV: p=0,0006

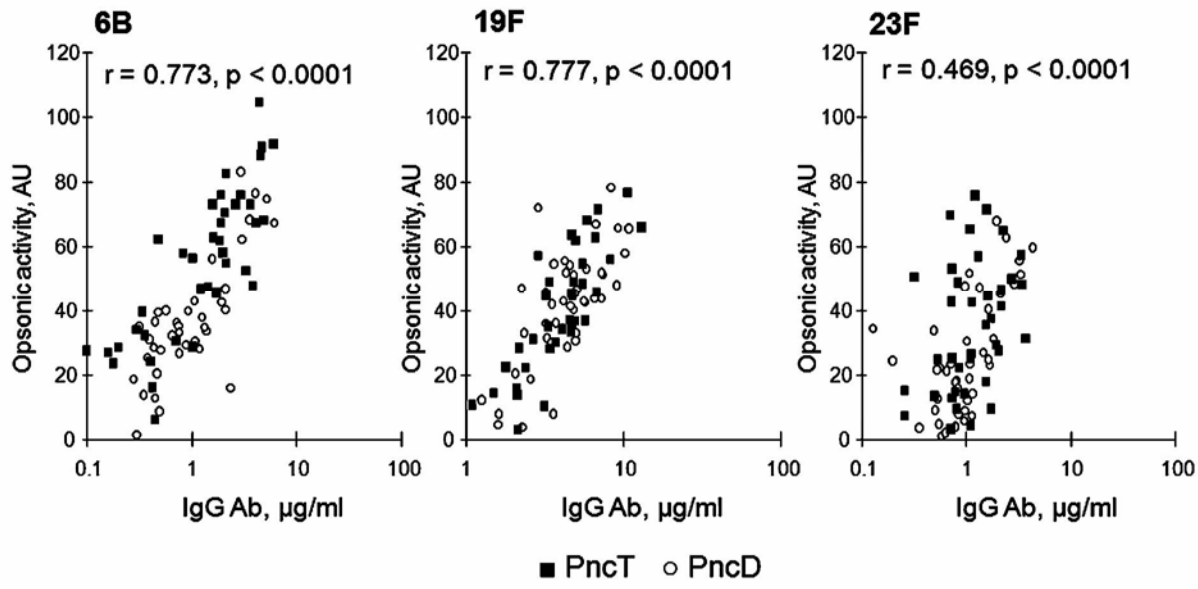
OPA was measured at 7 and 14 months for the three serotypes and because of low values for serotype 23F, it was measured in addition at 3 months to evaluate the immune response. Significant opsonophagocytic activity was measured at 7 months after the

[4] Abstracts: I. Jonsdottir, **STh Sigurdardottir**, G Vidarsson, G Ingolfsdottir, Th Gudnason, K Davidsdottir, S Kjartansson, KG Kristinsson og O Leroy. Pneumococcal conjugate vaccines elicit functional antibodies in infants. 27th Scandinavian Society for Immunology Meeting, Turku, Finland, May 24 – 27, 1996. Scand J. Immunol 1996, 43:710

[5] I.Jonsdottir, **S.Th.Sigurdardottir**, G.Vidarsson, G.Ingolfsdottir, Th.Gudnason, K.Davidsdottir, S.Kjartansson, K.G.Kristinsson and O. Leroy. Functional Activity of Antibodies Elicited by Octavalent Pneumococcal Polysaccharide Conjugate Vaccines, PncT and PncD. ICAAC, Toronto, Ontario, Canada, September 28 – October 1, 1997

three infant vaccinations for all three serotypes. When compared to the 3 month value the response to serotype 23F was significant; for PCV8-DT from OPA GMT 17,0 to 22,9 AU (p=0.0482) and for PCV8-TT from 14,9 to 24,3 AU (p=0.0051). Only for serotype 6B was there a difference between the vaccine formulations at 7 months, PCV8-TT resulting in higher OPA compared with PCV8-DT (p=0.0005). Opsonic activities correlated significantly with the levels of IgG and reflecting the highest IgG values the highest OPA was measured after the PPV23 booster in the PCV8-TT group for all three serotypes. Similar correlation between IgG levels and OPA was observed within the PCV8-DT and PCV8-TT groups for all the serotypes, demonstrated in Figure 4.

Figure 4. Opsonic activity after primary vaccinations with 8-valent PCV8-DT or PCV8-TT



The figure shows the serotype specific IgG vs. OPA for each individual. Both PCV8-DT and PCV8-TT groups are presented in the same graphs. Statistics shown is for both groups combined.

Avidity maturation

To investigate further the quality of the vaccine induced antibodies, avidity was evaluated at 14 months of age. Table 9 shows the mean avidity indexes and 95% CI of

IgG antibodies against serotypes 6B, 9V, 18C and 23F at 14 months, one month after the booster vaccination.

Table 10. Avidity of PCV8-DT or PCV8-TT induced-IgG antibodies against serotypes 6B, 19F and 23F

		At 14 months, post-booster			
Vaccine Serotype		**PCV8-DT-PCV8-DT**	**PCV8-DT-PPV23**	**PCV8-TT-PCV8-TT**	**PCV8-TT-PPV23**
6B	**Mean**	3.2	2.3	2.6	2.6
	95% CI	(2.87;3.53)	(2.08;2.52)	(2.36;2.84)	(2.29;2.91)
19F	**Mean**	3,1	2,9	3,2	2,8
	95% CI	(2.91;3.29)	(2.96;3.44)	(2.98;3.42)	(2.55;3.05)
23F	**Mean**	3,1	2,8	3,7	2,7
	95% CI	(2.79;3.41)	(2.55;3.05)	(3.46;3.94)	(2.42;2.98)

Unpublished data from Trial II. The table shows the mean and 95% CI for avidity index (AI) of IgG antibodies at 14 months after booster vaccination at 13 months. Avidity was not performed after primary vaccinations.

In this 8-valent PCV study (Trial II) the PCV8-DT conjugate booster tended to induce higher avidity to serotype 6B, significantly different from the PPV23 booster at 13 months (p=0,05) and the PCV8-TT booster tended to induce higher avidity antibodies to serotype 23F, significantly different from the PPV23 vaccine (p=0,01). For serotype 19F which induced much higher antibody levels than the other serotypes, the avidity was similar in all groups.

Further investigation of the protective capacity of the infant sera was performed using an *in vivo* mouse model.

Protective in vivo efficacy of post -vaccination sera (Paper III).

The mouse model was used to test randomly selected post- booster serum from 59 children who had been vaccinated with 8-valent PCV8-TT at 3, 4 and 6 months of age and given booster with either PCV8-TT or PPV23 at 13 months. Each serum sample was used to passively immunize two mice, three hours before intranasal challenge with either pneumococcal serotype 6B or 6A or a saline control. The serum protected mice against bacteraemia and/or lung infection caused by intranasal challenge with serotype 6B and 6A strains. Protective infant serum samples had significantly higher serotype-specific IgG, IgG1 and IgG2 levels and opsonic activity than did non-protective serum

samples (Paper III, table 1 and figure 1). The protective level to either serotype was approximately 1 μg of specific IgG antibodies injected per mouse (corresponding to approximately 0.3 μg/mL serum). In the sera used in this protection experiment a strong relationship was found between opsonophagocytic activities of the sera measured *in vitro* and serum IgG and IgG1 anti 6B levels, whereas OPA did not correlate with IgM levels or IgG avidity (Paper III, table 3). Similar rate of protection against serotype 6B was seen by sera from children boosted with the PCV8-TT PCV and PPV23.

When challenged with serotype 6A, a significant cross-protection was obtained by the passive immunization compared with the negative control after 24 hours (16/16 mice in the control group were infected compared with 22/44 mice immunized with post-vaccination serum). Compared with the non-protective sera the protective sera had significantly higher GMC IgG anti-6A levels, 0,24 AU/mL (95%CI 0,17-0,34) and 0,81 AU/mL (95%CI 0,39-1,65), respectively, as well as higher OPA (Paper III, table 1). As seen for serotype 6B, the IgM or avidity did not correlate with protection. The IgG anti-6A correlated with OPA ($r=0.47$; $p<0.0001$). The protective serum from infants boosted with PPV23 had higher GMC IgG anti-6A than protective sera from infants boosted with the PCV8-TT (1,46 vs. 0,28 μg/mL, respectively; $p=0.0105$), but the OPA, IgM levels or AI did not differ. At comparable GMC IgG anti-6A for protective (0,23 AU/mL) and non-protective (0,22 AU/mL) sera ($p=0.87$), the OPA was higher in the protective sera (9,7 vs. 6,8 AU; $p = .03$) while IgG avidity or IgM levels did not seem to contribute to protection in same samples.

The infant mouse model showed that the protection was strongly serotype specific and correlated with 6A and 6B IgG levels and OPA when challenged with respective serotypes. The level of IgG anti-6A correlated with 6B protection but not vise versa and the OPA for each serotype did not correlate with the IgG level of the other serotype. These results demonstrated that PCV8-TT induced anti-6B antibodies in infants that protect mice against invasive disease caused by the homologous serotype and by the cross-reacting serotype 6A.

Taken together, both of the octavalent pneumococcal conjugates, PCV8-DT and PCV8-TT, were safe and immunogenic in infants The PCV8-DT induced a better primary responses to serotypes 3, 9V and 18C, whereas PCV8-TT induced a better response to serotype 4. The two conjugates induced comparable immune responses to the other serotypes. The booster induced good booster IgG responses in all vaccine groups indicating a successful priming (Paper IV). Interference between DT protein carrier was observed in antibody responses to Hib conjugated to the same carrier ptotein. A functional capacity of vaccine induced antibodies was demonstrated by OPA and *in vivo* in mouse protection model.

Based on the results from Trial II and similar trials an 11-valent mixed diphtheria and tetanus pneumococcal conjugate vaccine was designed to provide the optimal immune response to each serotype. In the 11-valent formulation serotypes 1, 4, 5, 7F, 9V, 19F and 23F were conjugated to tetanus protein and serotypes 3, 14 and 18C and 6B to diphtheria toxoid. Although significant responses were obtained by conjugating with either Tetanus protein or Diphtheria toxoid and functional capacity could be demonstrated, the antibody responses were low to the poorly immunogenic pneumococcal serotypes; 6B, 9V, 18C and 23F. In an attempt to increase the T-cell help and therefore the antibody responses to these serotypes polysaccharides, they were conjugated to both TT and DT which led to the next clinical trial.

Do two carrier proteins enhance the antibody responses to the poorly immunogenic serotypes? The immunogenicity of two 11-valent pneumococcal conjugates, PCV11-F3 and PCV11-F3bis

Trial IV, unpublished data.

The PCV11-F3 vaccine contained serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F conjugated to either tetanus protein or diphtheria toxoid, whereas PCV11-F3bis contained the same serotypes in same formulation, but with both carrier proteins for serotypes 6B, 9V, 18C and 23F (For study design, see Table 5).

Antibody responses (Unpublished results from Trial IV[6,7]).

The results from this trial showed that both vaccine formulations induced significant serotype specific IgG responses to all serotypes. When comparing IgG responses induced by the two formulations at 7 months of age, no benefit was observed from the double carrier formulation for any of the four serotypes and indeed the single carrier formulation tended to give higher IgG levels at that age (Table 11). Before booster immunization at 13 months, serotype 6B specific IgG level was higher in the PCV11-F3bis group (p=0.002) and resulted in higher post booster level at 14 months (p=0.0001). Pre-booster levels for all the other serotypes were comparable between the vaccine groups. Out of the 4 double carrier serotypes, 18C also showed higher booster IgG response to PCV11-F3bis (p=0.0014) but the remaining serotypes, 9V and 23F were comparable as were the rest of the serotypes (Table 12).

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[6] Abstract: **S Sigurdardottir**, Þ Gudnason, KG Kristinsson, S Kjartansson, K Davídsdóttir, G Ingólfsdóttir, M Yaich, O Leroy, I Jónsdóttir. Safety And Immunogenicity Of Two Different Formulations Of 11-Valent Pneumococcal Polysaccharide Conjugate Vaccines, F3 And F3bis In Healthy Icelandic Infants. The 2nd ISPPD, Sun City, S-Africa, 19-23 March 2000 (abstract).

[7] Abstract: **ST Sigurdardottir**, T Gudnason, KG Kristinsson, S Kjartansson, K Davidsdottir, G Ingolfsdottir, M Yaich, O Leroy, I Jonsdottir: Do Two Carrier Proteins for the Less Immunogenic Serotypes Improve the Immune Response to the 11-Valent Pneumococcal Conjugate Vaccine? The 40th Interscience Conference on Antibmicrobial Agents and Chemotherapy, Canada in September 2000 (Abstract G-50).

Table 11. Serotype-specific IgG levels and proportions with indicated antibody concentrations at 7 months, after primary vaccinations with 11 valent PCV11-F3 or PCV11-F3bis.

Serotype		1	3	4	5	6B	7F	9V	14	18C	19F	23F
µg/mL (95% CI)	PCV11-F3*	2.67 (2.20; 3.24)	3.98 (3.37; 4.70)	4.17 (3.46; 5.02)	1.86 (1.53; 2.26)	0.82 (0.58; 1.14)	3.96 (3.35; 4.69)	1.95 (1.61; 2.37)	2.71 (2.12; 3.47)	1.87 (1.58; 2.21)	6.20 (4.78; 8.05)	1.32 (0.96; 1.82)
	PCV11-F3bis**	2.14 (1.68; 2.73)	3.39 (2.74; 4.19)	3.69 (2.80; 4.85)	1.68 (1.29; 2.18)	1.02 (0.71; 1.47)	4.01 (3.25; 4.95)	1.66 (1.29; 2.14)	2.20 (1.52; 3.20)	1.90 (1.48; 2.44)	3.99 (2.77; 5.75)	1.16 (0.84; 1.62)
% > 0.15 µg/mL	PCV11-F3	100	100	100	100	89	100	100	99	100	100	95
	PCV11-F3bis	100	100	100	100	88	100	99	95	97	100	92
% > 0.3 µg/mL	PCV11-F3	100	100	100	100	82	100	97	97	99	99	90
	PCV11-F3bis	97	100	99	96	85	100	96	90	97	95	85
% > 1.0 µg/mL	PCV11-F3	92	97	99	77	46	99	86	82	83	94	62
	PCV11-F3bis	85	94	88	64	47	94	74	65	80	79	59

Unpublished results from Trial IV. The table shows geometric mean concentration of IgG in µg/mL at 7 months, after primary vaccinations at 3, 4 and 6 months of age. Rate of responders is shown as percentage above 0.15 µg/mL (% ≥0.15), 0.3 µg/mL (≥0.3) and 1.0 µg/mL (% ≥1.0) for each formulation, PCV11-F3 (N=72) and PCV11-F3bis (N=71).

*PCV11-F3 formulation: *S. pneumoniae* purified capsular polysaccharides serotypes 1, 4, 5, 7F, 9V, 19F and 23F conjugated to tetanus protein and 3, 6B, 14 and 18C conjugated to diphtheria toxoid.

**PCV11-F3bis formulation: *S. pneumoniae* purified capsular polysaccharides serotypes 1, 4, 5, 6B, 7F, 9V, 18C, 19F and 23F conjugated to tetanus protein and serotypes 3, 6B, 9V, 14, 18C and 23F conjugated to diphtheria toxoid, (both carrier proteins for serotypes 6B, 9V, 18C and 23F).

Table 12. Serotype-specific IgG levels and rate of responders at 14 months, after booster vaccinations with 11-valent PCV11-F3 or PCV11-F3bis

Serotype		1	3	4	5	6B[a]	7F	9V	14	18C[b]	19F	23F
GMC µg/mL (95% CI)	PCV11-F3	8.30 (6.78; 10.2)	4.17 (3.46; 5.03)	8.00 (6.66; 9.62)	7.55 (6.41; 8.90)	3.07 (2.26; 4.16)	6.85 (5.85; 8.03)	3.77 (3.12; 4.55)	6.45 (4.95; 8.39)	2.13 (1.71; 2.65)	31.2 (24.9; 39.0)	4.86 (3.70; 6.40)
	PCV11-F3bis	5.9 (4.73; 7.36)	2.74 (2.25;3.34)	8.60 (6.90;10.7)	6.66 (5.36;8.28)	6.97 (5.38;9.03)	7.75 (6.37;9.44)	4.20 (3.42;5.15)	6.47 (4.72;8.85)	3.39 (2.89;3.99)	24.4 (17.9;33.30)	4.45 (3.29;6.02)
% > 1 µg/mL	PCV11-F3	100	96	100	100	80	100	94	96	73	99	90
	PCV11-F3bis	97	91	99	94	94	100	94	90	96	96	88

*Unpublished results from Trial IV. The table shows geometric mean IgG concentration and 95% CI

**PCV11-F3 formulation: *S. pneumoniae* purified capsular polysaccharides serotypes 1, 4, 5, 7F, 9V, 19F and 23F conjugated to tetanus protein and 3, 6B, 14 and 18C conjugated to diphtheria toxoid.

***PCV11-F3bis formulation: *S. pneumoniae* purified capsular polysaccharides serotypes 1, 4, 5, 6B, 7F, 9V, 18C, 19F and 23F conjugated to tetanus protein and serotypes 3, 6B, 9V, 14, 18C and 23F conjugated to diphtheria toxoid, (both carrier proteins for serotypes 6B, 9V, 18C and 23F).

[a)] PCV11-F3 vs PCV11-F3bis: p=0.0001, [b)] PCV11-F3 vs. PCV11-F3bis: p=0,0014.

Protective capacities.

Opsonophagocytosis

Functional capacity of the vaccine induced antibodies was measured *in vitro* for 5 serotypes by OPA, at 7 months, one month after the primary vaccinations. Besides the four serotypes with both Diphtheria toxoid and Tetanus protein carrier, serotype 19F was also tested (identical in both formulations). Highest OPA was observed for serotype 9V, approximately 50% adult OPA level in the PCV11-F3 group and 42,5% in the PCV11-F3bis group. The lowest OPA was observed for serotype 23F with 22% and 25% for PCV11-F3 and PCV11-F3bis, respectively and around 30% adult activity for 6B and 18C in both groups (Table 13).

Table 13 . Opsonophagocytic activity at 7 months after primary vaccination with either PCV11-F3 or PCV11-F3bis.

Serotype		PCV11-F3	PCV11-F3bis
6B	**GMT**	36.3	37.7
	(95% CI)	(31.1;42.4)	(31.9;44.6)
9V	**GMT**	50.8	42.5
	(95% CI)	(42.3;61.0)	(34.5;52.2)
18C	**GMT**	32.8	34.2
	(95% CI)	(27.3;39.5)	(28.5;40.9)
19F	**GMT**	32.5	29.3
	(95% CI)	(27.3;38.7)	(24.1;35.6)
23F	**GMT**	26.1	22.6
	(95% CI)	(21.1;32.2)	(18.1;28.2)

Unpublished results from Trial IV. The table shows the OPA GMT and 95% conficence interval for 5 serotypes. The results are expressed as percentage of post-vaccination sera from 10 healthy adults.

No difference was found in opsonophagocytic activity between the two formulations for any of the five serotyped tested, which is in agreement with comparable IgG responses.

Avidity maturation

To investigate further the quality of the vaccine induced antibodies, avidity was measured. Table 14 summarizes the geometric mean avidity indexes of IgG antibodies

against the same serotypes as OPA (6B, 9V, 18C, 19F and 23F) at 7 months and at 14 months, one month after the booster vaccination.

Table 14. Avidity of IgG antibodies after the primary and booster vaccination with 11- valent PCV11-F3 or PCV11-F3bis.

		11-valent D- and/or T- conjugate			
		At 7 months, post primary		At 14 months, post-booster	
Vaccine Serotype		**PCV11-F3**	**PCV11-F3bis**	**PCV11-F3**	**PCV11-F3bis**
6B	GMAI	0.52	1.31	0.678	2.22
	95% CI	(0.45; 0.60)	(1.10; 1.56)	(0.56; 0.83)	(1.83; 2.69)
9V	GMAI	1.27	2.95	1.11	2.65
	95% CI	(1.10; 1.48)	(2.55; 3.41)	(0.97; 1.27)	(2.26; 3.11)
18C	GMAI	1.11	2.19	1.25	2.55
	95% CI	(0.99; 1.26)	(1.93; 2.48)	(1.12; 1.40)	(2.27; 2.87)
23F	GMAI	0.92	2.49	0.84	2.03
	95% CI	(0.76; 1.11)	(2.09; 2.97)	(0.71; 1.0)	(1.65; 2.51)
19F	GMAI	1.11	3.33	1.25	2.69
	95% CI	(0.98; 1.27)	(2.97; 3.72)	(1.09; 1.44)	(2.32; 3.13)

Unpublished results from Trial IV. The table shows geometric mean avidity index (GMAI) and 95% CI of antibodies against serotypes 6B, 9V, 18C, 19F, and 23F induced by PCV11-F3 and PCV11-F3bis formulations of the pneumococcal conjugate vaccine at 7 months after primary vaccinations at 3, 4 and 6 months and at 14 months after booster vaccination at 13 months.

In response to the 11-valent conjugates the double carrier formulation PCV11-F3bis induced antibodies with higher avidity to all five serotypes measured, both after primary and booster vaccinations. That applied also to serotype 19F, although the 19F conjugate was identical in both formulations, namely conjugated to tetanus protein in equal quantities of protein and polysaccharide.

Thus, using both diphtheria toxoid and tetanus protein as carriers for the poorly immunogenic pneumococcal serotypes in the same vaccine formulation does not enhance the primary IgG responses to these serotypes, but higher avidity might indicate better quality of the antibodies. This was however not reflected in higher opsonic activity.

How many primary doses are needed in infancy to generate sufficient antibody responses and immunological memory? Safety and immunogenicity of CRM_{197}-conjugated pneumococcal-meningococcal C combination vaccine

Paper V.

Antibody responses.

Currently licensed pneumococcal and meningococcal conjugate vaccines are registered for three primary doses for children within 6 months of age. In Iceland and the Nordic countries as well as a few other European countries, infant vaccinations are administered in two doses at age 3 and 5 months with a booster dose around 12 months of age. We asked if routine primary vaccination with a pneumococcal conjugate vaccine could be applied to our current vaccination schedule without compromising the immunity and protection provided. Several countries in Europe have already introduced PCV vaccination in two primary doses with a booster (236-240).

We compared in a randomized trial the immunogenicity of a three dose infant schedule with a two dose schedule using a combination vaccine of CRM_{197} conjugated 9-valent pneumococcal and a meningococcal type C vaccine (9vPnCMnCC) (For study design, see Table 5). At 6 months of age the 9vPnCMnCC induced significant IgG antibodies to all vaccine components in both groups ($P<0.001$, for all serotypes). Three doses of 9vPncMnCC induced higher antibody GMCs to all but serotypes 1 and 9V that were comparable between the schedules. The difference between the 2- and 3-dose scheduling was most significant for 6B and 23F ($p<.001$), that also showed lower percentage of responders above 0.35 μg/mL (6B, 23F) and above 0.5 μg/mL (6B) (Paper V, table 2).

The booster vaccination after 3 primary doses tended to result in higher geometric mean IgG, but there was not a significant difference in antibody levels or rate of responders to any of the serotypes (Paper V, table 4). Blood sample obtained from a subgroup of children one week after the booster showed significant memory responses to the PPV23 toddler dose in both the 2- and 3-dose groups (Paper V, table 5). Meningococcus C

specific IgG GMC was lower at 6 months after two compared with tree primary doses, however, the SBA was comparable (Paper V, table 3).

This trial showed that the 9vPncMnCC vaccine induced lower IgG antibody responses when given in two primary doses compared to three primary doses. However, more than 94% of vaccinees reached the estimated protective antibody levels (>0.35µg/mL) to 7 out of 9 serotypes. The rate of responders above 0.35µg/mL for serotype 6B was 62% and 86% for the 2- and 3-dose groups and for serotype 23F, 82% and 90%, respectively. Comparable immunological memory was generated, whether the 9vPnCMnCC was given in two or three primary doses, as shown by rapid and strong response to one PPV23 booster at 12 months of age.

The results from this trial show that two doses of 9vPncMnCC induce lower IgG primary responses than three doses, however with significant and brisk memory responses induced by PPV23 at one year, indicating successful priming with both schedules.

Generation of immunological memory.

Paper IV, V and unpublished data.

Generation of immunological memory is of major importance in protective vaccine response and is one of the advantages introduced with the protein conjugation of pneumococcal polysaccharides in the new generation of pneumococcal vaccines.

A memory response is characterized by a rapid rise in IgG antibodies (and sometimes IgA) after subsequent polysaccharide challenge, whereas a primary IgG response is slower. We investigated the kinetics of antibody responses to booster vaccination in four studies.

Short term immunological memory.

In trial II, a booster vaccination at 13 months was randomized to be administered with either PCV8-DT or PCV8-TT, according to the formulation received during primary vaccinations, or with the PPV23 vaccine. Post booster sample was obtained 4 weeks later. A strong booster response was observed with significant increase of serotype specific IgG responses to all vaccine serotypes in all four groups (Paper IV, table 3). The polysaccharide booster induced significantly higher IgG responses than the conjugate booster and infant priming with the PCV8-TT induced higher booster responses to PPV23 than PCV8-DT priming. These results indicated successful immunological priming with significant memory responses at 13months of age.

In the phase I trial, trial III (Unpublished data[8]), on safety and immunogenicity of the 11-valent PCV11-F3bis formulation, twenty toddlers were vaccinated with one dose of

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[8]Abstract: I Jonsdottir, G Ingolfsdottir, E Saeland, K Davidsdottir, M Yaich, O Leroy, **S Sigurdardottir**: A Single Dose of Pneumococcal Conjugate Vaccine Elicits Functional Antibodies and Induces Memory in Toddlers. The 40th Interscience Conference on Antibmicrobial Agents and Chemotherapy, Canada, September 2000 (Abstract # 43).

PCV11-F3bis at 17 months of age. The immune response was evaluated before and after 4 weeks. Ten months after the PCV11-F3bis vaccination, 9 toddlers were given a booster with a full dose of PPV23 to evaluate memory responses by measuring IgG antibodies before and one week. As shown in Table 15 and Figure 5, the primary IgG response was significant for all 11 serotypes after the primary vaccination.

Table 15. IgG responses to PCV11-F3bis after one dose at 17 months of age and booster with PPV23 at 27 months of age.

	PCV11-F3bis priming at 17 months of age (no = 20)					**PPV23 booster at 27 months of age (no = 9)**				
	GMC			Rate of responders		GMC			Rate of responders	
Serotype	µg/mL pre	µg/mL post	Increase p-value*	% >0.35	% >1.0	µg/mL pre	µg/mL post	Increase p-value*	% >0.35	% >1.0
1	0.22	0.74	<0.001	70	45	0.43	29.21	<0.001	100	100
3	0.21	2.97	<0.001	100	95	0.59	19.65	0.008**	100	100
4	0.19	4.64	<0.001	100	95	1.95	41.53	<0.001	100	100
5	0.49	1.11	0.002	95	50	1.95	44.90	<0.001	100	100
6B	0.19	0.32	0.016	55	20	1.40	9.74	0.001	89	89
7F	0.27	4.14	<0.001	95	90	2.22	14.31	0.004	100	100
9V	0.34	2.61	<0.001	95	90	2.46	17.94	0.002	100	100
14	0.39	0.58	0.01	60	15	2.76	18.43	0.001	100	100
18C	0.16	1.88	<0.001	95	80	0.64	15.69	<0.001	100	100
19F	0.45	1.10	<0.001	85	55	3.61	32.59	0.012	89	78
23F	0.25	1.44	<0.001	80	65	1.51	12.23	<0.001	100	100

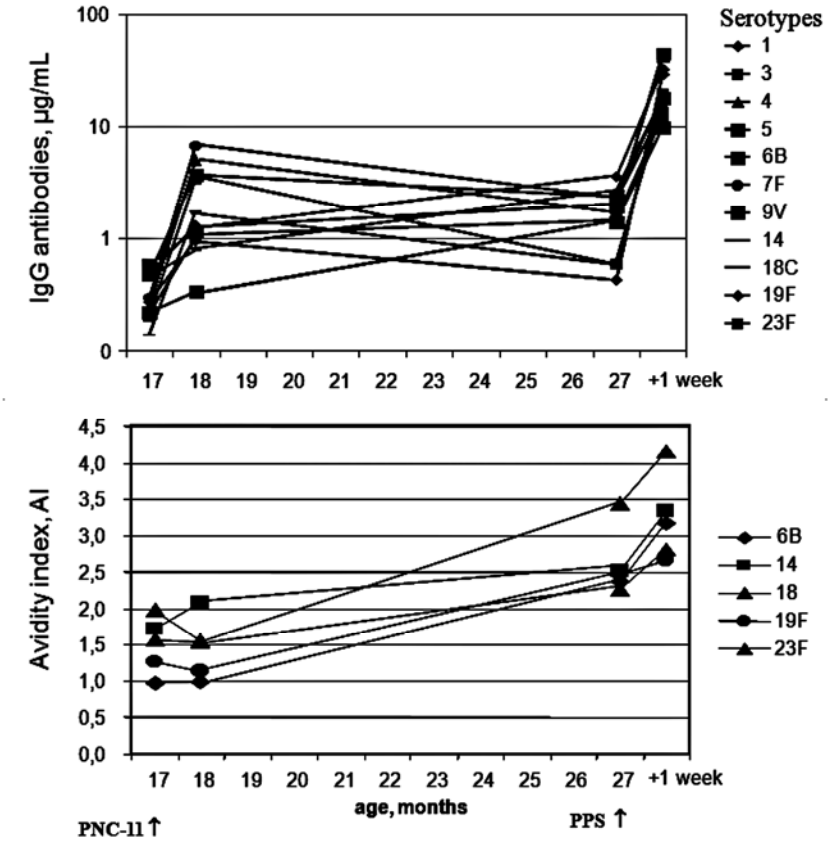
Unpublished results from trial III. The table shows GMC IgG in µg/mL, and rate of responders; % $\geq$0.35µg/mL and $\geq$1.0 µg/mL, 4 weeks after one dose of PCV11-F3bis at 17 months (left panel) and 1 week after full dose PPV23 booster at 27 months of age (right panel).

* Paired T-Test on log transformed data

** Wilcoxon Signed Rank Test on log transformed data when normality test failed

Furthermore, the polysaccharide booster 10 months later elicited rapid memory IgG responses and avidity maturation observed already one week after the booster, demonstrating a successful priming and memory generation with one dose at 17 months of age (Figure 5, unpublished data).

Figure 5. IgG responses and avidity maturation after PCV11-F3bis vaccination at 17 months of age and a booster with PPV23 at 27 months of age.



Unpublished results from trial III. The upper panel shows serotype specific IgG GMC for each of the 11 serotypes in the PCV11-F3bis and the lower panel the avidity index for five serotypes. Measurements were done before and 4 weeks after primary vaccination at 17 months and before and one week after a polysaccharide booster 10 months later at 27 months of age.

Opsonic activity to five serotypes was also measured and found to increase significantly at 4 weeks after the immunization at 17 months (serotypes 6B, 14, 18C, 19F and 23F; ($p<0.001$, 0.001, 0.001, 0.016 and 0.005, respectively), which correlated with the IgG levels for serotypes; 9V ($r=0.476$, $p=0.034$), 18C ($r=0.793$, $p<0.001$), 19F ($r=0.856$, $p<0.001$) but not for serotype 6B ($r=0.08$, $p=0.737$) in which the correlation was

significant with the avidity index (r = 0.585, p = 0.007), not observed for the other serotypes

In trial V (Paper V), on the 9vPnCMnCC we similarly investigated IgG memory responses to the PPV23 booster at 12 months when half of the subjects received the conjugate and the other half received PPV23. Serum samples obtained from a subgroup of children 7 days after the booster showed a significant increase in IgG to all the serotypes (Paper V, Table 5). This rapid increase in IgG was observed whether the primary vaccinations had been given in two or three doses at 3 and 5 or 3, 4 and 5 months, indicating a successful memory generation with both schedules, although the two doses induced lower primary IgG responses to 7 serotypes out of 9, measured at 6 months.

Thus, by using PPV23 booster vaccination we have demonstrated effective priming by immunization of infants with three doses of PCV-TT or PCV-DT and two or three vaccinations with Pnc9MnCC. We also demonstrated that one dose of PCV11-DT/TT at 17 months primes for booster responses 10 months later.

Long term memory. (Paper IV and unpublished data[9]).

The objective of the next study was to assess the long term persistence of memory IgG responses, 6 years after booster vaccination, and to evaluate if a polysaccharide booster could exhaust or deplete memory cells generated by conjugate vaccines in infancy. Children who at 3, 4 and 6 months of age were vaccinated with PCV8-TT pneumococcal conjugate vaccine in 1995-6 were randomized to receive booster with the same PCV8-TT conjugate or a full dose PPV23 at 13 months (Paper IV). Thirty two of these children participated in this additional study on immunological memory in at 7 years of age, when they were challenged with a fractional dose of PPV23 (1/10 of a dose; 2.5 µg/serotype). The serotype specific IgG response was analysed according to the type of

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[9] Abstract: S. T. Sigurdardottir, K. Davidsdottir, I. Jonsdottir. Effect of a Pneumococcal Polysaccharide (PPS) Booster on Immunological Memory of Children Vaccinated With a Pneumococcal Conjugate (Pnc) in Infancy. Oral precentation at the 43rd Interscience Conference on Antibmicrobial Agents and Chemotherapy, Chicago, USA, September 2003 (Abstract # G-2049).

booster at 13 months and compared to unvaccinated controls. Out of the 32 children 17 had received PCV8-TT booster (PCV8-TT group) and 15 had received PPV23 (PPV23-group) at 13 months. Nine previously unvaccinated age-matched controls were challenged and antibody responses measured. IgG responses to 11 serotypes were measured, including all eight PCV8-TT vaccine types and the non-vaccine serotypes 1, 5 and 7F. Blood was obtained on days 0, 7 and 28. The IgG results for each serotype in all the groups at all three time points are summarized in Table 16 and furthermore illustrated in Figure 6. Pre-challenge IgG antibodies to 19F were significantly higher in both booster groups than in unvaccinated controls and also for serotype 6B in the PCV8-TT group. In one week, a rise in geometric mean (GMC) IgG was observed in both vaccine groups, significant for serotypes 4, 6B, 9V, 18C and 23F in the PCV8-TT group and serotypes 6B and 14 in the PPV23 group, whereas no significant responses were detected to the 8 PCV8-TT serotypes in the previously unvaccinated controls. The differences between PCV8-TT and PPV23 groups were not significant except for serotype 6B pre-challenge IgG levels which were higher in the PCV8-TT group.

The IgG subclasses were analysed for five serotypes, the PCV8-TT vaccine serotypes 6B, 19F and 23F and serotypes 1 and 5 not contained in the PCV8-TT vaccine, but present in the PPV23 vaccine. After the toddler doses, 6 years earlier, at 14 months, both IgG1 and IgG2 levels were higher in response to PPV23 booster at 13 months compared to the PCV8-TT induced responses for the vaccine serotypes. However the pre challenge IgG1 levels at 7 years of age tended to be higher in the PCV8-TT than the PPV23 group for serotypes 6B ($p<.05$) and 19F (ns). IgG1, IgG2 and the ratios of IgG1/ IgG2 are presented in Table 17 (Unpublished data[10]). From 14 months to 7 years of age the ratio of IgG1/IgG2 for serotype 6B decreased significantly for both vaccine groups ($p<.001$) ending in higher proportion of IgG2 in pre challenge blood sample at 7 years. Although the PCV8-TT group responded with higher IgG1 than the PPV23 group the difference

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[10] Abstract: Sigurdardottir ST, Davidsdottir K, Jonsdottir I. IgG Subclass Responses to Pneumococcal Polysaccharide (PPS) Booster 6 years after Pneumococcal Conjugate (Pnc) Vaccination in Infancy. Effect of PPS Booster at 13 Months. Presented as poster at the 4th ISPPD, Helsinki, May 9 – 13th 2004.

was not significant between the two vaccine groups and both had significantly higher IgG1/IgG2 ratio than the previously unvaccinated controls at all time points ($p<.001$) (Table 17).

For serotype 19F, the ratio of IgG1/IgG2 decreased similarly between age 14 months and 7 years ($p<.001$ in both groups), but with no difference between the vaccine groups. Compared with the unvaccinated controls during the PPV23 challenge at 7 years, the IgG1/IgG2 ratio was significantly higher for the PCV8-TT ($p<0.05$, before challenge at 7 years, $p<.01$ one week later and $p<.05$ after 4 weeks) while the ratio in the PPV23 group was comparable to the controls at all time points. In response to the PPV23 challenge at 7 years, the IgG1/IgG2 ratio was >1 for both serotype 6B and 19F in the conjugate group, while it was <1 in the PPV23 group, as well as in the unvaccinated controls. Although the same pattern was observed for serotype 23F the levels were much lower. There was a significant change in IgG1/IgG2 ratio from 14 months of age to 7 years ($p<.001$) in both groups, but with no difference between PCV8-TT and PPV23 groups at either age. With the low response observed after the PPV23 challenge at 7 years, no differences were observed in the IgG1/IgG2 ratio between any of the groups at any time of the responses.

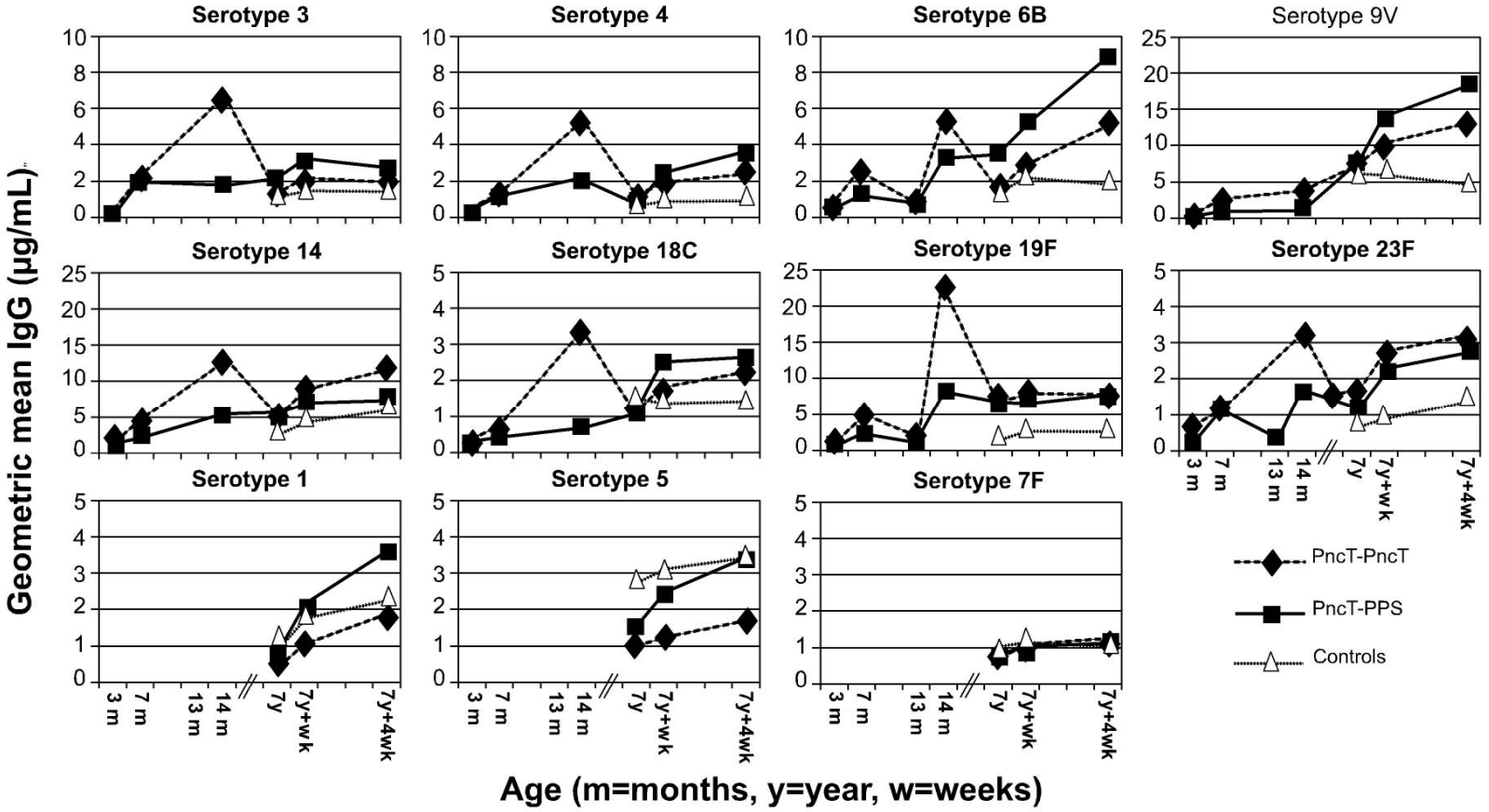
Although antibodies to serotypes 1 and 5 were very low in the two vaccine groups at 14 months of age the majority was of the IgG1 subclass, giving a ratio of IgG1/IgG2 between 3 and 15. At 7 years of age that ratio had reversed with an IgG2 dominating response to the PPV23 challenge at 7 years. With the very low levels of antibodies mounted against the two non-vaccine serotypes no difference was observed between the two vaccine groups. Compared to the unvaccinated controls at 4 weeks after the PPV23 challenge at 7 years, the PCV8-TT group showed a higher IgG1/IgG2 ratio to serotype 1 ($p<0.05$). Similarly for serotype 5, the PPV23 group showed higher ratio at 4 weeks after PPV23 challenge. For the non-vaccine serotypes, IgG2 dominated the responses in all the groups (Table 17).

Table 16. Long term immunological memory as demonstrated by IgG responses to fractional PPV23 at 7 years.

		PCV8-TT booster at 13 months N=16			PPV23 booster at 13 months N=14			Unvaccinated controls N=9		
Serotype		**Before**	**1 week**	**4 weeks**	**Before**	**1 week**	**4 weeks**	**Before**	**1 week**	**4 weeks**
PCV8-TT vaccine serotypes										
3	GMC	2.26	3.06	2.75	1.33	1.99	1.60	1.30	1.65	1.61
	95% CI	(1.46-3.08)	(2.28-3.83)	(2.07-3.43)	(0.52-2.14)	(1.18-2.79)	(0.83-2.37)	(0.31-2.29)	(0.78-2.51)	(0.74-2.49)
4	GMC	0.72	2.28	3.73	1.24	1.74	2.23	0.80	1.03	1.11
	95% CI	(-0.10- 1.54)	(1.48-3.09)	(2.96-4.5)	(0.27-2.21)	(0.85-2.63)	1.42-3.05)	(-0.19-1.79)	(0.17-1.89)	(0.37-1.85)
6B	GMC	3.68	5.19	8.88	1.83	2.79	5.30	1.74	2.06	2.23
	95% CI	(2.99-4.37)	(4.50-5.88)	(8.09-9.66)	(0.99-2.67)	(1.95-3.62)	(4.29-6.32)	(1.01-2.46)	1.32-2.81)	(1.47-2.89)
9V	GMC	7.62	14.33	18.40	6.94	10.72	13.37	6.17	5.62	4.84
	95% CI	(6.82-8.43)	(13.58-15.07)	(17.7-19.10)	(6.03-7.86)	(9.92-11.53)	12.64-14.11)	(5.42-6.92)	(4.84-6.39)	(4.06-5.62)
14	GMC	5.38	7.00	7.48	4.46	9.36	12.04	2.90	4.33	5.86
	95% CI	(4.63-6.13)	(6.27-7.73)	(6.80-8.15)	(3.69-5.23)	(8.60-10.12)	(11.35-12.74)	(2.23-3.57)	(3.54-5.13)	(4.96-6.76)
18C	GMC	1.09	2.52	2.78	1.28	1.88	2.12	1.55	1.46	1.45
	95% CI	(0.24-1.94)	(1.80-3.25)	(2.04-3.52)	(0.46-2.09)	(1.04-2.73)	(1.32-2.91)	(0.56-2.54)	(0.52-2.40	(0.64-2.25)
19F	GMC	7.11	7.03	8.26	7.63	8.52	7.57	1.43	2.70	2.88
	95% CI	(6.29-7.94)	6.11-7.94)	(7.32-9.19)	(6.90-8.36)	(7.81-9.23)	(6.74-8.39)	(0.56-2.30)	(1.82-3.58)	(2.03-3.74)
23F	GMC	1.29	2.22	2.82	1.61	2.72	3.11	0.84	0.81	1.31
	95% CI	(0.45-2.14)	(1.41-3.02)	(1.96-3.68)	(0.73-2.49)	(1.86-3.58)	(2.24-3.99)	(-0.38-2.05)	(-0.20-1.81)	(0.22-2.41)
Non PCV8-TT serotypes										
1	GMC	0.37	1.01	1.86	0.73	2.07	3.64	1.11	1.74	2.20
	95% CI	(-0.46-1.21)	(0.30-1.72)	(1.18-2.54)	(-0.18-1.63)	(1.35-2.80)	(2.90-4.37)	(0.36-1.87)	(1.03-2.44)	(1.59-2.81)
5	GMC	0.99	1.24	1.67	1.55	2.42	3.37	2.71	3.04	3.33
	95% CI	(0.10-1.88)	(0.37-2.10)	(0.85-2.48)	(0.55-2.55)	(1.68-3.16)	2.70-4.05)	(1.93-3.81)	(2.28-3.81)	(2.63-4.03)
7F	GMC	0.65	0.85	1.03	0.76	0.88	1.05	1.05	1.20	1.22
	95% CI	(-0.01-1.31)	(0.13-1.57)	(0.24-1.83)	(-0.04-1.57)	(0.12-1.65)	(0.30-1.80)	(0.26-1.83)	(0.33-2.07)	(0.36-2.09)

Unpublished data from additional study to Trial II. The table shows GMC IgG and 95% CI for eight serotypes included in the PCV8-TT vaccine (PCV8-TT serotypes) and three serotypes not in the PCV8-TT vaccine (Non PCV8-TT serotypes) before, one week and 4 weeks after a fractional dose of PPV23 in a group of children at 7 years of age, who received PCV8-TT pneumococcal conjugate in infancy and either same conjugate (PCV8-TT booster) or PPV23 (PPV23 booster) at 13 months of age. The third group did not receive pneumococcal conjugate in infancy (Unvaccinated controls)

Figure 6. Serotype specific IgG responses to fractional PPV23 challenge in 7 year old children who were vaccinated with PCV8-TT in infancy and boosted with PCV8-TT or PPV23 at 13 months of age.



Unpublished results from additional study to Trial II. The figure shows the serotype specific GMC (µg/mL) at each immunogenicity time point in the same individuals before and after primary vaccination, before and after booster vaccination (serotype 6B, 19F and 23F but only after for the other serotypes) and before, 1 and 4 weeks after fractional PPV23 dose at 7 years of age. Vaccine serotypes are 3, 4, 6B, 9V, 14, 18C, 19F and 23F. Non-vaccine serotypes are serotypes 1, 5 and 7F. Note different scale on Y-axis.

PCV8-TT in infancy and PCV8-TT booster (PCV8-TT (PCV8-TT-PCV8-TT: closed squares) or PCV8-TT priming and PPV23 booster (PCV8-TT-PPV23: closed diamonds) or did not receive a pneumococcal conjugate in infancy (controls: open triangles).

Table 17. Kinetics of IgG subclass responses after a fractional dose of PPV23 in children who received PCV8-TT in infancy and either PCV8-TT or PPV23 at 13 months of age or did not receive pneumococcal conjugate in infancy.

	PCV8-TT booster at 13 months N=16				PPV23 booster at 13 months N=14				Unvaccinated controls N=9		
	14 months	1/10 PPV23 challenge at 7 years of age			14 months	1/10 PPV23 challenge at 7 years of age			1/10 PPV23 challenge at 7 years of age		
	Post toddler dose	Before	1 week	4 weeks	Post toddler dose	before	1 week	4 weeks	before	1 week	4 weeks
					Serotype 1						
IgG1	0,18 (-0.60-0.97)	0.14 (-0.55-0.82)	0.46 (-0.21-1.14)	0.89 (0.19-1.60)	0.77 (-0.03-1.56)	0.22 (-0.60-1.04)	0.71 (-0.53-1.95)	1.12 (-0.14-2.38)	0.15 (-1.14-1.44)	0.42 (-0.78-1.62)	0.55 (-0.59-1.70)
IgG2	0.04 (-0.90-0.97)	0.83 (0.00-1.67	0.88 (0.04-1.72)	1.04 (0.22-1.86)	0.05 (-1.03-1.12)	1.12 (0.17-2.07)	1.51 (0.22-2.80)	1.93 (0.69-3.18)	1.68 0.46-2.90)	1.75 0.53-2.97)	1.97 0.80-3.14)
IgG1 / IgG2	5.23	0.17	0.52	0.86	15.79	0.20	0.47	0.58	0.09	0.24	0,28
					Serotype 5						
IgG1	0.44 (-0.29-1.17)	0.07 (-0.78-0.91)	0.19 (-0.55-0.93)	0.30 (-0.44-1.03)	0.65 (-0.04-1.33)	0.08 (-0.89-1.06)	0.31 (-0.92-1.54)	0.47 (-0.75-1.69)	0.12 (-1.02-1.27)	0.16 (-0.99-1.31)	0.28 (-0.88-1.45)
IgG2	0.14 (-0.69-0.98)	1.60 (0.82-2.38)	1.72 0.92-2.51)	1.82 1.02-2.61)	0.16 (-0.62-0.94)	1.93 (1.07-2.78)	1.87 (0.59-3.16)	2.44 (1.21-3.67)	2.99 (1.79-4.18)	3.33 (2.16-4.50)	3.46 (2.34-4.58)
IgG1 / IgG2	3.12	0.04	0.11	0.16	3.96	0.04	0.17	0.19	0.04	0.05	0.08
					Serotype 6B						
IgG1	3.44 (2.64-4.24)	1.49 (0.72-2.25)	2.91 (2.06-3.76)	3.65 (2.74-4.55)	5.42 (4.67-6.16)	0.46 (-0.35-1.26)	1.23 (-0.11-3.09)	1.66 (0.22-3.09)	0.17 (-1.02-1.37)	0.21 (-0.99-1.41)	0.30 (-0.91-1.52)
IgG2	0.66 (-0.22-1.55)	1.87 (1.15-2.58)	2.31 (1.59-3.03)	2.86 (2.09-3.62)	1.64 (0.75-2.54)	1.23 (0.42-2.05)	1.34 (0.11-2.57)	1.98 (0.66-3.30)	2.06 (0.92-3.21)	2.45 (1.29-3.62)	2.08 (0.85-3.30)
IgG1 / IgG2	5.18	0.80	1.26	1.28	3.29	0.37	0.92	0.84	0.08	0.09	0.15
					Serotype 19F						
IgG1	11.75 (10.95-12.55)	5.20 (4.24-6.16)	7.23 (6.26-8.20)	7.77 (6.66-8.87)	34.87 (34.1-35.64)	3.98 (3.28-4.67)	5.13 (3.96-6.29)	5.19 (4.02-6.37)	1.07 (-0.19-2.33)	1.40 (0.18-2.61)	2.56 (1.36-3.77)
IgG2	1.21 (0.26-2.15)	3.76 (3.04-4.44)	4.13 (3.43-4.83)	4.04 (3.35-4.74)	4.68 (3.90-5.46)	5.58 (4.78-6.38)	6.00 (4.77-7.22)	6.70 (5.48-7.92)	3.91 (2.76-5.06)	4.28 (3.11-5.44)	4.99 (3.87-6.11)
IgG1 / IgG2	9.74	1.38	1.75	1.92	7.45	0.71	0.86	0.77	0.27	0.33	0.51
					Serotype 23F						
IgG1	1.48 (0.76-2.21)	0.52 (-0.45-1.49)	0.73 (-0.22-1.68)	0.75 (-0.16-1.67)	2.20 (1.37-3.04)	0.42 (-0.37-1.20)	0.72 (-0.55-2.00)	0.74 (-0.47-1.94)	0.23 (-1.15-1.61)	0.23 (-1.16-1.62)	0.36 (-1.02-1.73)
IgG2	0.21 (-0.66-1.08)	1.16 (0.4-1.91)	1.40 (0.68-2.12)	1.69 (0.95-2.43)	0.72 (-0.15-1.60)	1.27 (0;53-2.02)	1.58 (0.36-2.80)	1.81 (0.57-3.05)	1.13 (-0.25-2.51)	1.29 (-0.05-2.64)	1.38 (0.02-2.73)
IgG1 / IgG2	6.98	0.45	0.52	0.45	3.04	0.33	0.46	0.41	0.20	0.18	0.26

The table shows serotyped specific GMC and 95% CI (µg/mL) IgG1, IgG2 and IgG1/IgG2 ratio to five serotypes at 14 months og age after booster at 13 months and before, one and four weeks after a cjaæææmge wotj 1/10 of a PPV23 dose at 7 years of age. in a group of children who received PCV8-TT pneumococcal conjugate in infancy and either same conjugate (PCV8-TT booster) or PPV23 (PPV23 booster) at 13 months of age. The third group did not receive pneumococcal conjugate in infancy (Unvaccinated controls).

Thus, IgG1 subclass accounted for higher proportion of specific antibodies responses to serotypes 6B and 19F vaccine types in children who received PCV8-TT in infancy, whether they received a booster with PCV8-TT or PPV23 at 13 months, when compared to unvaccinated control group and responses to non-vaccine serotypes (NVT).

In summary, the study shows that children, immunized with PCV8-TT vaccine in infancy still have immunological memory to the vaccine serotypes at 7 years of age. This was shown by brisk and significant IgG responses to PCV8-TT serotypes elicited by a fractional PPS challenge which was not observed in unvaccinated controls. Compared with children who received a PPV23 booster at 13 months of age, the PCV booster recipients tended to respond with higher IgG and with a higher IgG1/IgG2 ratio to the vaccine serotypes.

The effect of pneumococcal conjugate vaccines at mucosal level.

Unpublished data from Trial I, II and IV.

<u>The effect of pneumococcal conjugate vaccines on nasopharyngeal colonization.</u>

The nasopharyngeal mucosa is the port of entry for mucosal and invasive infections. As a part the study design in three trials (Trial I, II and IV; Paper I and unpublished data), we obtained nasopharyngeal cultures (NPC) from the participants during the vaccination period and up to one and a half year beyond. The aim was to study the effect of PCV vaccinations on nasopharyngeal carriage. In two trials we compared two formulations of pneumococcal conjugates, namely 8-valent PCV8-DT and PCV8-TT (trial II) and the 11-valent PCV11-F3 and PCV11-F3bis formulations (Trial IV). No differences in nasopharyngeal carriage were noted between the two groups in either of the trials. Therefore, the two vaccine arms in each of the two trials were analyzed together as "vaccinees".

In Trial I we immunized infants with the mono-valent 6B-TT PCV at 3, 4, 6 and 18 months (Group A) or 7, 9 and 18 months (Group B) and obtained nasopharyngeal swabs for culturing at each visit to the health care centre, at 3, 4, 6, 7, 10, 18, and 24 months in group A and at 7, 9, 10, 18, and 30 months in group B. Altogether 293 nasopharyngeal swabs were obtained from 40 infants and children. Pneumococci were isolated in 130 (14%) and serogroup 6 from 16 infants. Numbers were too low to make any conclusion on the vaccine effect on nasopharyngeal colonization (Paper I, Figure 3).

In Trial II on 8-valent PCV8-DT vs. PCV8-TT (Unpublished data[11]) a total of 628 nasopharyngeal cultures were obtained from the 82 vaccinees, thereof 254 (40%) were positive for pneumococci. In 177 cases it was a new acquisition leaving 80 cases (30%) with recurrent or persistent colonization. From the 40 unvaccinated controls, 296 cultures were obtained with 139 (47%) positive for pneumococci of which 100 were new acquisition, leaving 39 cases (28%) with a persistent or recurrent colonization. The difference between the total positive cultures in vaccinees and controls was not significant with p=0.0641 (RR=0.8612; 95% CI 0.7385-1.005). The total serotype distribution is shown in Figure 7, top panel, with serotype 6A being the most prevalent one, followed by serotype 6B, 19F, 23F, 14, 19F, 15, 17, 11, 16, 33, 3, 18C, 9V, 17 and 23A in descending order. Few additional serotypes not illustrated on the figure, were cultured in less than 0.4% of the swabs including serotypes 10, 13, 18F, 22, 12, 1, 4 and a few nontypeable pneumococci. As shown in the middle panel in same figure, the prevalence was similar in the vaccinated group and the controls with the exception of serotype 19F which were cultured significantly less often from the vaccinees (P<0.0001) which also was reflected in fewer new 19F acquisition (p=0.003) as shown in Figure 7, lower panel. Serotype 19A did not change significantly with time with total of 17 cultures from 12 children during the study period from the vaccinees and six cultures from five control children.

A decrease in VT pneumococci was observed after third vaccination compared with control group at same age and in same geographical area, but not vaccinated with PCV (p=0.0203). A similar trend was observed at 14 months, 1 month after the booster vaccination (Figure 8).

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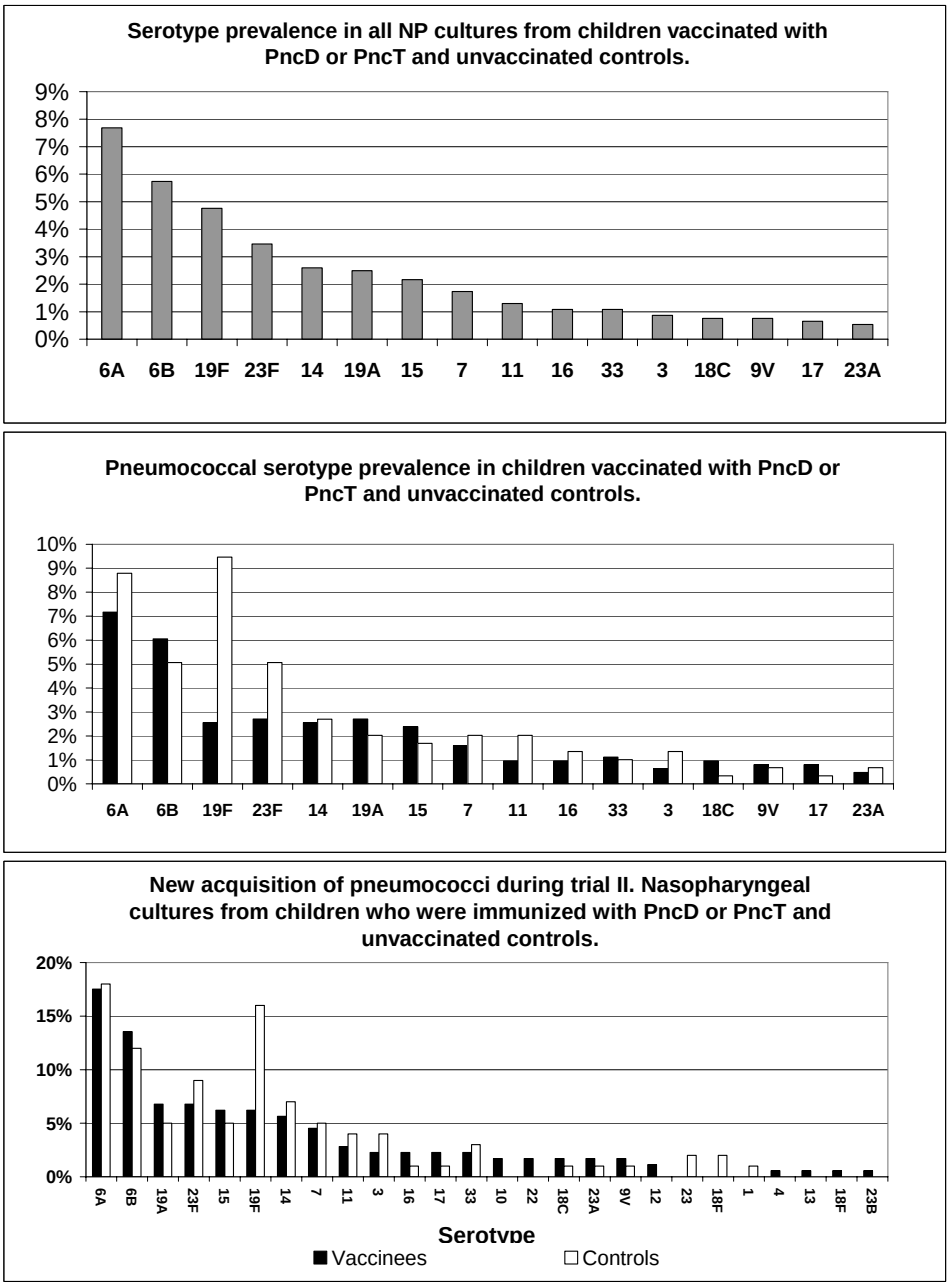
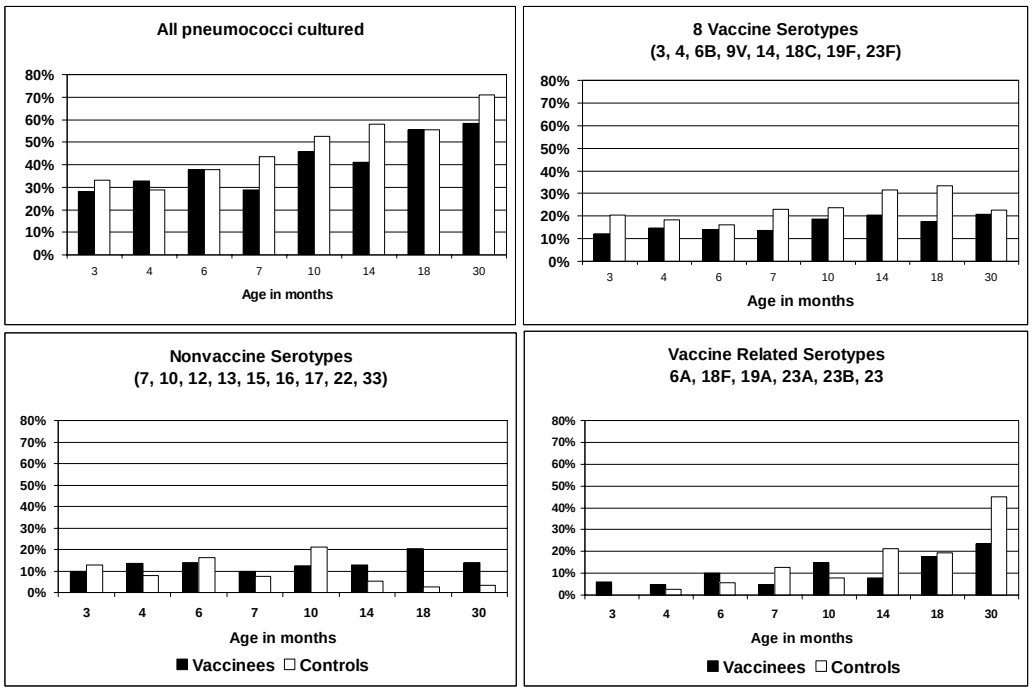
[11] Abstract: K.G.Kristinsson, **S.Th.Sigurdardottir**, Th.Gudnason, S. Kjartansson, K.Davidsdottir, O.Leroy and I. Jonsdottir. Effect of Vaccination with Ocatvalent Protein Conjugated Pneumococcal Vaccines on Pneumococcal Carriage in Infants. ICAAC, Toronto, Ontario, Canada, September 28 – October 1, 1997 [Abstract].

Figure 7. Serotype distribution of pneumococci cultured from nasopharynx of children vaccinated with 8-valent Pnc-D or Pnc-T pneumococcal conjugate vaccine.



Unpublished data from trial II. The figures shows the collective data form nasopharyngeal cultures obtained from vaccinees and controls at 3, 4, 6, 7 10, 14, 18 and 30 months. The top panel shows the percentage prevalence of each serotype out of the total 924 nasopharyngeal swabs obtained from both vaccinated (N=82) and unvaccinated (40) children. The middle panel shows the prevalence of each serotype in total cultures obtained from vaccinated children (N 628) and unvaccinated controls (N=296). The bottom panel shows the rate of new acquisition of each serotype during the whole trial period.

Figure 8. Pneumococcal nasopharyngeal colonization in children vaccinated with 8-valent Pnc-D or Pnc-T PCVs.



Unpublished results from trial II. The figure shows the percentage point prevalence of any pneumococci (upper left), vaccine type pneumococci (upper right), nonvaccine serotypes (lower left) and vaccine related serotypes (lower right), in children vaccinated with PCV8-DT or PCV8-TT (both groups analyzed together) at 3, 4, 6 and 13 months of age (closed columns) and PCV non-vaccinated control group at same age (open columns). Nasopharyngeal culture swabs were obtained at each visit, 3, 4, 6, 7, 10, 14, 18 and 30 months.

Although not significant at each age, collectively at all ages, the vaccine serotypes (VT) (3, 4, 6B, 9V, 14, 18C, 19F, 23F) were cultured less often from vaccinees compared with the controls, (p=0.0112) and when analysed after infant priming vaccinations (p=0.0203) or after booster vaccination (p=0.05). If serotype 6A was included with vaccine types this association was even stronger with p=0.004 for all the visits. In the analyses some subjects were counted more than once, as many children were repeatedly colonized. The vaccine-related serotypes (VRT), (mainly 6A and 19A, a few 23A and sporatic cases of 18F, 23B and 23) were more often cultured from the control group both after the infant vaccinations (p=0.0474) and after the booster vaccinations (p=0.0175). The increase in VRT at 30 months was mostly accounted for by serotype 6A. At 30 months, the point prevalence of VRT

was higher in the control group (p=0.0364) and a trend was observed at 14 months (p=0.0647). The nonvaccine serotypes (NVT) (7, 10, 12, 13, 15, 16, 17, 22, 33 and nontypable), were collectively more prevalent in the vaccinees after the booster vaccination (p=0.016) with significant point prevalence difference at 18 months (p=0.019).

The 11-valent PCV11-F3 and PCV11-F3bis vaccines were administered at age 3, 4, 6 and 13 months in Trial IV (Unpublished data[12]). As part of the study protocol, comparing the advantage of mixing two carriers for serotypes 6B, 9V, 18C and 23F, NP cultures were obtained from 146 infants at 4 and 7 months and from 143 infants at 10 and 14 months of age. In addition, follow-up cultures were obtained from 125 of the participants at 18 months and 108 at 24 months. At 24 months, unvaccinated controls (N=105) were recruited from age-matched playmates of PCV recipients, who were in the same group childcare at that time. A total of 808 nasopharyngeal swabs were obtained from Trial IV vaccinees out of which 357 (44%) were pneumococcus positive with 125 VTs (35%), 143 VRT (40%) and 88 NVTs (25%). Serotype 6A counted for 25% of pneumococci cultured. The serotype distribution within the control group was comparable to the vaccinees at 24 months of age with 57% vs. 61% colonized with pneumococci, respectively. Thereof 24% and 24% were colonized with VTs, 22% and 25% with VRTs, 11% and 12% with VT for controls and vaccinees, respectively.

No differences were observed between the vaccinees and the unvaccinated controls at 24 months of age, allowing merging of the groups to analyse the serotype prevalence. As demonstrated in Figure 9, the most prevalent serotype was serotype 6A, followed by 23F, 19F, 6B, 19A, 14, 23A, 15, 9N, 16, 9V, 11, 22 and 3 in descending order. One – three cases of each of the following serotypes were also cultured but not illustrated on the figure; 17, 23, 13, 6, 7, 9, 18F, 23B, 33.

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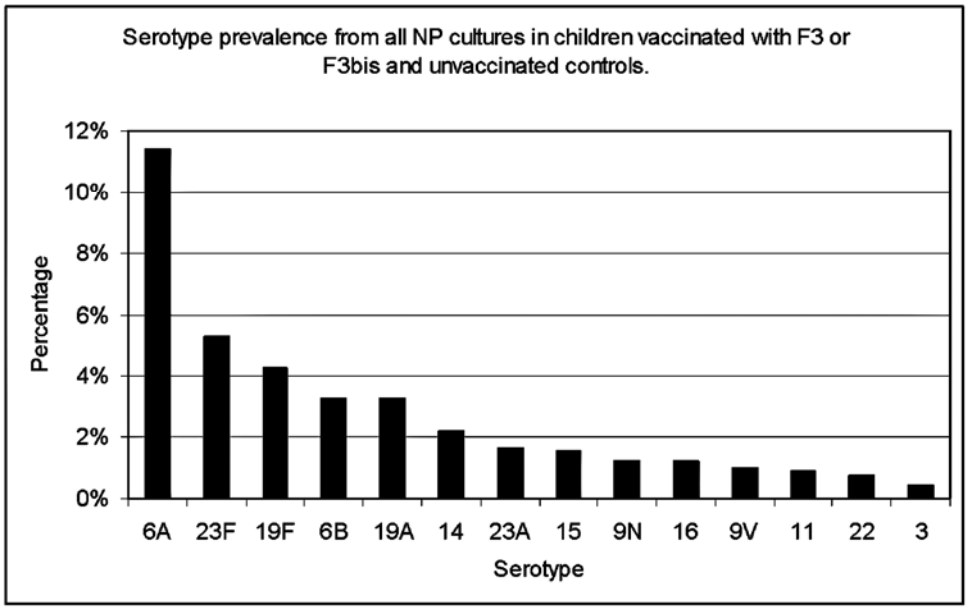
[12] Abstract: S.T. Sigurdardottir, I. Jonsdottir, T. Gudnason, K. Davidsdottir, S. Kjartansson, M. Yaich, K.G. Kristinsson: Effect of 11-valent penumococcal vaccine (Pnc) on pneumococcal colonization in children at 2 years of age. Presented at the 41st Interscience Conference on Antibmicrobial Agents and Chemotherapy, Chicago, USA 2001 (Abstract # G86).

Figure 9. Serotype distribution of pneumococci cultured from nasopharynx of children during participation in trial IV and from unvaccinated controls at two years of age.



Unpublished results from trial IV and amended study. The figure shows the prevalence of pneumococcal serotypes cultured from all NP swabs (N=808 cultures swabs) collected at 4, 7, 10, 14, 18 and 24 months of age, from children vaccinated with either PCV11-F3 or PCV11-F3bis and from unvaccinated controls at 24 months of age (N=105).

The presence and serotype distribution of pneumococci-positive NP cultures in the subjects of the PCV11-F3 and PCV11-F3bis groups was similar and no difference was observed for VT or NVT (Table 18, unpublished data).

Vaccine-type nasopharyngeal colonisation decreased between 7 and 10 months before remaining stable (PCV11-F3bis group) or increasing (PCV11-F3 group) between 10 and 14 months. As there were no significant differences in NP carriage between the vaccine groups, the data were merged in order to gain power for analysis of the trend by time. The VRT, mostly 6A, increased significantly between 14 and 18 months (p=0.0387) as well as NVT (p=0.0025), which after that remained stable up to 30 months in both cases. The VT, increased at slower pace, and by 24 months of age the VT pneumococci had also increased compared to the colonization rate at 14 months (p=0.0054).

Table 18. Percentage of nasopharyngeal samples colonized by a vaccine or a non-vaccine type of *S. pneumoniae* in Trial IV.

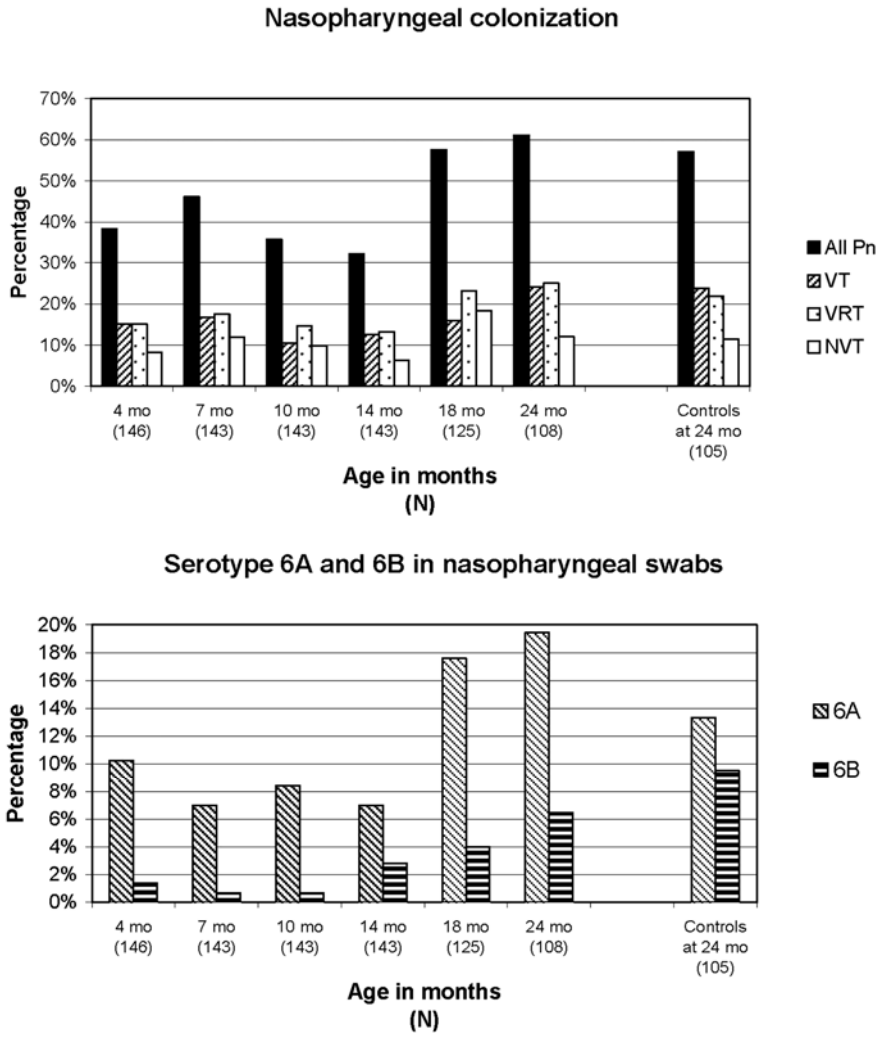
	4 months	7 months	10 months	14 months
PCV11-F3 group				
Vaccine type	**13.7%**	**16.4%**	**9.6%**	**13.9%**
Major (6B, 9V, 18C, and 23F)	6.9%	6.9%	5.5%	6.9%
Other	6.9%	9.6%	4.1%	6.9%
Non-vaccine type	**19.2%**	**19.2%**	**19.2%**	**16.7%**
PCV11-F3bis group				
Vaccine type	**16.4%**	**16.4%**	**11.3%**	**11.3%**
Major (6B, 9V, 18C, and 23F)	6.9%	11.0%	5.6%	5.6%
Other	9.6%	5.5%	5.6%	5.6%
Non-vaccine type	**20.6%**	**26.0%**	**22.5%**	**16.9%**

Unpublished data from trial IV. The table shows comparison of NP carriage between the two vaccine groups at 4 time points. Major serotypes were conjugated to both protein carriers (Diphtheria toxoid and Tetanus protein) in the PCV11-F3bis formulation. No differences were between the groups (Fisher's exact test).

Some decrease was observed in NP pneumococci between age 7 and 14 months but subsequently increased significantly at age 18 months. This was mostly due to an increase in serotype 6A, as shown in Figure 10. The trial design of this study did not allow analysis of new acquisitions of pneumococci due to infrequent nasopharyngeal culturing.

When compared to 105 control children, matched for age and day-care centre at 24 months of age, no differences were observed in nasopharyngeal colonisation by pneumococci, VT, NVT or individual serotypes. Neither was there a differences in colonisation by *H. influenzae* and *M. catarrhalis* between vaccinees and controls at that age. No statistical differences were found between the 2-year old vaccinees and the unvaccinated age mached controls.

Figure 10. Pneumococcal nasopharyngeal colonization in children vaccinated with PCV11-F3 or PCV11-F3bis and in an age matched control group.



Unpublished results from trial IV. The figure shows pneumococcal nasopharyngeal colonization in children vaccinated with PCV11-F3 or PCV11-F3bis, 11-valent PCV at 3, 4, 6 and 13 months, during and 10 months beyond the study period and in an age matched control group at 24 months of age.

Percentage point prevalence of pneumococci in nasopharynx. The upper panel shows the percentage of children colonized with any pneumococci, vaccine serotype (VT), vaccine related serotype (VRT) or nonvaccine serotype (NVT) pneumococci at each time point. Significant increase in rate of colonization was observed between age 14 and 18 months for all pneumococci ($P<0.0001$), VRT ($P = 0.04$) and for 6A separately ($P<0.01$), Fisher's exact test. The lower panel shows the change in serotype 6A and 6B analysed separately.

The increase of VTs at 18 months may indicate prevention of colonization by VTs in the vaccinees before, which subsequently disappears with increasing age and time from vaccination and is comparable with unvaccinated controls at 2 years of age.

The next step was to analyse the relationship between frequencies of nasopharyngeal colonization and serotype specific IgG responses to the vaccine serotypes in PCV11-F3 and PCV11-F3bis vaccines.

Nasopharyngeal colonization and relationship with serotype specific IgG antibody responses

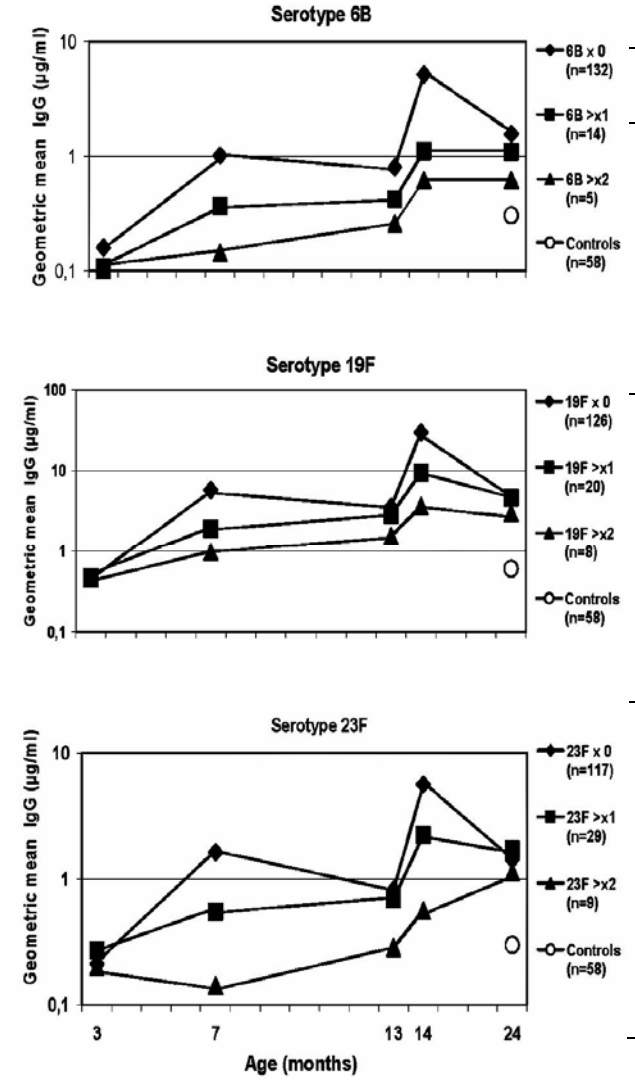
In Trial I and II, on 6BTT and PCV8-DT or PCV8-TT it was observed that infants with nasopharyngeal cultures repeatedly positive for serotype 6B had poor serum IgG responses to the vaccine (Unpublished data[13]). This prompted us to look in Trial IV if there was a relationship between poor vaccine responses and NP colonization. We compared the PCV serotype-specific IgG response in children carrying vaccine serotypes in the nasopharynx to that in children with no positive culture for the serotype in question.

As described above, 146 infants received PCV11-F3 or PCV11-F3bis at 3, 4, 6 and 13 mo. NP samples were cultured at 4, 7, 10, 14, and 18 and 24 mo. Antibodies to vaccine serotypes were measured by ELISA at 3, 7, 13, 14 and 24 months. One hundred and five control children of the same age and at same day-care setting were recruited at 2-years of age out of whom the parents of 58 consented to have blood sample drawn from their child for antibody measurements. Of the 146 vaccinated infants, 14, 20 and 29 infants had positive NP cultures for the PCV serotypes 6B, 19F and 23F, respectively, at least at one visit between 4 and 24 months. As shown

[13] Abstract: Sigurveig Þ. Sigurdardottir, Þorolfur Gudnason, Gunnhildur Ingolfsdottir, Karl G Kristinsson, Katrin Davidsdottir, Sveinn Kjartansson, Odile Leroy and Ingileif Jonsdottir. Pneumococcal Colonization And Specific IgG Antibodies In Vaccinated Infants, ESPID, Crete, Greece, May 1999.

in Figure 11, these infants had lower post-primary vaccination and post-booster IgG levels compared to the vaccinated infants with

Figure 11. Serotype-specific IgG GMC in children who were colonized with serotype 6B, 19F or 23F, or not.



Serotype Colonized	N	7 months GMC P-value	14months GMC P-value
6B Never colonized	**132**	1.01	5.22
6B Colonized ≥1	**14**	0.37 <0.01	1.11 <0.001
6B Colonized ≥2	**5**	0.14 0.002	0.62 <0.001
19F Never colonized	**126**	5.83	29.97
19F Colonized ≥1	**20**	1.93 <0.001	9.59 <0.001
19F Colonized ≥2	**8**	0.98 <0.001	3.71 <0.001
23F Never colonized	**117**	1.63	5.62
23F Colonized ≥1	**29**	0.55 0.002	2.23 <0.001
23F Colonized ≥2	**9**	0.14 <0.001	0.56 <0.001

The figure shows GMC (µg/mL) in subjects colonized not at all (◆), at least once (■) or more than once (▲) during the trial. Unvaccinated controls were evaluated at 24 months (○).

Comparison between subjects colonized and not colonized was done with a t-test on a log transformed data, shown in the table on right which shows also GMC (µg/ml) in subjects never colonized, colonized at least once (+≥1) or more than once (+≥2) during the trial.

negative NP cultures at each visit. The vaccinated group had significantly higher IgG GMC to all 11 serotypes at 24 months of age when compared to unvaccinated

controls, as shown in Figure 11 for three serotypes, 6B, 19F and 23F. These three serotypes were the only ones with enough positive NP cultures to allow statistical analysis on serospecific antibody levels in the subgroup with positive NP cultures. (Unpublished data[14]).

The conclusion from this study was that infants who were colonized with serotypes 6B, 19F or 23F during the trial period were found to have lower serotype-specific IgG compared with children who were never found to be colonized with same serotype. Repeated colonization with these serotypes seems in particular to be related to poor responses to the PCV.

Effect of pneumococcal vaccination on otitis media and antibiotic usage.

Additional study to trial IV, Unpublished data[15].

We have reported comparable nasopharyngeal carriage of VT and non-vaccine type (NVT) pneumococci in vaccinated and unvaccinated controls at 24 months, but higher serotype specific IgG levels in vaccinated infants.

Infants were vaccinated at 3, 4, 6 and 13 months with PCV11-F3/F3bis as described before. At 24 months, the clinical data for 64 children vaccinated with either PCV11-F3 or PCV11-F3bis and their 105 controls, was evaluated for history of physician diagnosed-otitis media, pneumonia, sinusitis, other infections and antibiotic usage by obtaining information from the parents for the preceding 6 months. The controls were unvaccinated playmates, attending the same day-care

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[14] Abstract: S.T. Sigurdardottir, K.G. Kristinsson, G. Ingolfsdottir, T. Gudnason, K. Davidsdottir, S. Kjartansson, M. Yaich, I. Jonsdottir. Nasopharyngeal (NP) carriage of vaccine serotype pneumococci is more common in children who respond poorly to the 11-valent pneumococcal (Pnc) conjugate. Presented at the 3rd International Symposium on Pneumococci and Pneumococcal Diseases, Anchorage Alaska May 5- 9, 2002.

[15] Abstract: S Th.Sigurdardottir, Th Gudnason, K Davidsdottir, S Kjartansson, KG Kristinsson, M Yaich, I Jonsdottir. Pneumococcal Conjugate Vaccine Reduces Otitis Media And Antibiotic Use In Children Between 18 And 24 Months. 3rd World Congress On Pediatric Infectious Diseases, Santiago, Chile, Nov. 19 – 23, 2002.

centre and same play-area as the index child and at comparable age (+/- 6 months). They had not received a prior pneumococcal vaccination and were recruited during the same time period as the 24-month visit of index children.

Median age was 24 months for both groups with 95% distribution of 23.5-25.2 and 18.2-28.9 months for vaccinees and controls, respectively. History of otitis media during the preceding 6 months was more common in the controls than vaccinees, 43% and 23%, respectively (p=0.0084, Fisher's exact test). (Table 19). The control group reported a higher number of antibiotic courses (0.9 vs. 0.63 /child, p<0.0001) and tympanostomy tubes, 20% vs. 8%, respectively (p=0.0465). The incidence of other infections was similar in both groups.

Table 19. History of physician-diagnosed acute otitis media between 18 and 24 months of age.

	Vaccinees (n)	Controls (n)	Odds ratio	p-value
Number	64	105		
Otitis media x 1-3	13	40	0.41 (0.20- 0.86)	0.017
Otitis media ≥ x 4	2	6	0.53 (0.10- 2.72)	0.712
All otitis media	15	46	0.39 (0.20- 0.79)	0.008
Tympanostomy tubes	5	21	0.339 (0.12- 0.95)	0.047

Unpublished data from amended study to trial IV. Vaccinees: Two year old children who were vaccinated with PCV11-F3 or PCV11-F3bis PCV at 3, 4, 6 and 13 months of age. Controls: Unvaccinated playmates at same age from same day-care.

The statistical analysis was done with Fisher's exact test.

The data suggests that PCV vaccination in infancy protects children against acute otitis media and leads to reduced antibiotic usage, a question which has to be answered in a lager group of vaccinees.

DISCUSSION

The results presented in this thesis involve the development of a safe and effective pneumococcal vaccine for the young with the long-term aim to decrease morbidity and mortality of those who are most susceptible to pneumococcal diseases. The safety and immunogenicity of six pneumococcal polysaccharide protein conjugate vaccines, administered to Icelandic infants concomitantly with their routine vaccinations were investigated in five clinical trials. We have compared safety and immunogenicity of different carrier proteins, different valences, induction of immunological memory in toddlers and 6 years after primary vaccination as well as the effects on nasopharyngeal carriage and otitis media. The trials have demonstrated that the vaccines are safe, causing no unacceptable side effects. They are immunogenic when they are administered concomitantly with routine infant vaccinations, inducing significant antibody responses and immunological memory. They do at least temporarily, tend to decrease the nasopharyngeal colonization of the vaccine serotypes and may influence the rate of otitis media and antibiotic usage.

Safety

The safety of pneumococcal conjugate vaccines has been studied extensively in several trials that all confirm the safety of the pneumococcal conjugate formulations. Local reactions including redness, swelling and indurations are usually mild and self-limiting (143, 205, 206, 291).

In the trials comparing two conjugate formulations (Trial II and IV), no differences were demonstrated in total local or total systemic side effects during the infant vaccinations. However, after booster vaccination the 8-valent PCV8-DT formulations caused fewer side effects than both the PCV8-TT formulation and the PPV23 in Trial II. The safety profiles of the two 11-valent mixed Diphtheria toxoid and Tetanus protein carrier formulations, PCV11-F3 and PCV11-F3bis, were similar in Trial IV. The rate of local and systemic reactions after the 8-valent and the 11-

valent DT/TT conjugates in our studies were similar to what has been previously published for 4- and 11- valent DT/TT conjugates (205-207). The 11-valent vaccines tended to induce higher rate of local reactions than the 8-valent vaccines. Although that may be due to a longer follow-up time, most of the local reactions subsided within 4 days. It may also be related to higher number of serotypes and therefore higher dose of carrier proteins in the 11-valent vaccine. In the Philippino efficacy study the nonadjuvanted PCV11-F3 vaccine was used (191).

The 3-dose schedule with the 9vPnCMnCC resulted in a higher number of side effects than the 2-dose schedule due to higher number of injections but at the same visit and age, there were no differences between the groups for the same vaccine.

When side effects due to the PCVs were compared with concomitant vaccines in Trial II and IV, the pneumococcal conjugate vaccines caused less local reactions. That difference may be related to the whole cell pertussis component administered as concomitant vaccine in Trial I, II and IV. There is a well documented difference of rate of local and systemic side effects between whole cell and acellular pertussis vaccine. The DTwP is known to cause five times more local side effects than DT vaccination and causes significantly more systemic side effects as well (185, 292). In Trial V, an acellular pertussis vaccine was used, but no comparison was done on local reactions with the concomitant vaccines, DTaP/PRP-T/IPV. However as shown in Table 3, a lower rate of systemic reactions was recorded than in the previous trials that used DTwP combinations. This was despite a longer follow-up period in Trial V, which was 7 days. In the KPNC trial, the PCV7 induced less side-effects than the concomitant DTwP-HbOC vaccine, while the booster dose, compared with an acellular DTaP, given separately with HbOC resulted in comparable side-effects (210). Other studies on the safety of 9vPnCMnCC vaccine have also shown that the vaccine is safe when administered at 2, 3 and 4 months, although the combination caused more systematic reactions than Meningococcal C CRM_{197} conjugate alone (229). The same combination vaccine administered mixed with HbOC or with HbOC in a separate injection was safe when administered at 2, 4 and 6 months (293). A study performed in The Gambia used the same pneumococcal conjugate formulation as in 9vPnCMnCC in Trial V at the age 2, 3 and 4 months. After the 2nd

dose the trial vaccine caused a little more local swelling and tenderness than the control vaccine IPV, but during other time points the side effects were similar. However, the PCV vaccination caused less local side effects than the concomitant vaccines including DTP (178). Other studies have reported less local reactions after CRM_{197} than after concurrent vaccines such as DTP, polio vaccine or meningococcal conjugate vaccine (294).

The PPV23 vaccine caused more local side effects than PCV in the two trials where it was randomized to be used for booster vaccination, Trial II on 8-valent PCV8-DT/PCV8-TT conjugate and Trial V on the 9-valent CRM197 conjugate. In Trial II the PCV8-DT and PCV8-TT caused 18% and 29% local reactions, respectively, while the PPV23 caused 44% and 50% in the PCV8-DT and PCV8-TT groups respectively. In Trial V the 9vPncMnCC caused local reactions in 34% in both the 2-dose and 3-dose group while the PPV23 caused 53% and 51%, respectively. This is in line with previously reported data on PCV vs. PPV23 (222). Other studies have reported primary vaccinations with PCV and a booster with PPV23 without a direct comparison, showing usually mild unspecific local reaction following the PPV23 vaccination (213).

Previous studies have mostly reported fewer reactions due to PCV vaccines compared with concomitant vaccines (143, 205). A new systematic review of the safety profile of pneumococcal conjugate vaccines articles, identified 42 published articles and two abstracts from searches of the PubMed, Cochrane Collaboration databases, bibliographic references and expert consultations within the field (192). The review indicated the safety of PCV7 with mild local side effects and mild fever but with increasing rate with number of injections (143, 194-196, 295) with the highest rate after the toddler dose. This trend was not observed for more severe reactions (195-197). In our studies on the DT and TT PCVs, a similar trend was observed but not for the 9vPnCMnCC-CRM_{197} conjugate. Some randomized trials have suggested that PCV7 may result in more local reactions than control vaccines such as Men C CRM_{197} conjugate (143) or hepatitis B vaccine (193). On the other hand a lower reactogenicity has also been reported (210). Two large placebo

controlled trials on 9-valent PCV in Africa demonstrated similar local reactions as for the control vaccines (272, 214).

Asthma leading to hospital admission was one of the severe adverse events (SAE) reported in all our studies. In a prospective birth cohort study in Iceland published 2000, 34% of 20 month old children had been diagnosed with asthma and although none of them had severe or moderately severe asthma, reactive airway disease was very common in this age group (296). In the KPNC efficacy trial, hospitalization due to asthma within 60 days from immunization was recorded more frequently in the group receiving PCV7 than in the control group; however, it was comparable with asthma prevalence outside the hospital setting which did not indicate an association with the PCV7 (143, 297). One follow-up study to the KPNC efficacy trial, a post marketing assessment of uncommon events following PCV7 (presented as an abstract), found after adjusting for potential confounders (N= 65.927) a 20% increase in reactive airway disease, associated with hospitalization (RR, 1.23; P<0.02) among infants who received PCV7 compared with a historical cohort who received Hib vaccine (N=35.549) (298). In South African trial of a 9-valent PCV (PCV9; same as in the 9vPnCMnCC vaccine in Trial V) in children with and without HIV, the PCV9 recipients were more likely to be hospitalized for virus induced asthma (p=0.009), during first 8 days after PCV9 vaccination compared with children not vaccinated. These infections were primarily due to RSV and raised the question if the infants are at higher risk for infection immediately after PCV9 vaccination (214). Other studies have investigated possible role of immunizations in the development of asthma. In another African randomized efficacy trial on the same vaccine, in The Gambia, no association with asthma was observed (272). A big cohort study involving 167.240 children out of whom 18.407 children (11.0%) developed asthma found no association between diphtheria, tetanus and whole cell pertussis vaccine, oral polio vaccine or measles, mumps and rubella vaccine and the risk of asthma. A weak associations found for Hib and hepatitis B vaccines was considered to be at least partially accounted for by health care utilization or information bias RR=1.07 (0.71 to 1.60) for Hib and 1.09 (0.88 to 1.34) for hepatitis B vaccine (299).

In our studies, one child had pneumococcal serotype 7F bacteremic infection, only one week after 9vPnCMnCC vaccination. Since serotype 7F was not in the 9vPnCMnCC vaccine used, this adverse

event was considered probably not related to vaccination. In the above South African efficacy trial on PCV9 the vaccinees were transiently at increased risk for confirmed viral pneumonia during the first 8 days (p=0.03). The authors hypothesize on possible rational for the increased risk of viral pneumonia, one being some B-cell depletion following the PCV9 stimulation and the other being maternal antibody consumption by complex formation with the PCV9 antigens. The RSV infection may then have rendered the PCV9 recipients, more susceptible to pneumococcal pneumonia, but that was not confirmed. The same observation was made in the PCV11-F3 efficacy trial in the Philippines, where an increased rate of pneumonia was observed in the 11PCV group compared to the placebo group within 7 days and 28 days of the first dose, and also within 28 days of the second dose (191).

In none of the studies presented in this thesis were severe adverse events related to the trial vaccines. Considering the extensive work in recording safety parameters, relatively little safety data are presented in this thesis. All our trials demonstrated that the pneumococcal conjugate formulations tested were safe when used in infants and toddlers. The differences between the trials in terms of vaccine composition, carrier proteins and type and number of concomitant vaccines makes it impossible to compare the adverse events caused by different trial PCV formulations tested in the studies that this thesis is based on. In addition the time length of safety recording varied between studies.

Overall, our results along with previously published data on the same vaccine formulations as well as other formulations of pneumococcal conjugate vaccines have demonstrated that the PCVs are very safe vaccines with the potential to prevent morbidity and mortality caused by the pneumococcal serotypes contained in the vaccines.

Immunogenicity

PCVs induce protective immune responses in infants.

The concept of polysaccharide protein conjugation for the development of vaccines against the pneumococcus was derived from successful development of vaccine

against *Haemophilus influnzae* type b, (reviewed in (300)) which has literally eliminated invasive diseases caused by the organism in societies where the vaccine was introduced for routine use in infants (163, 301). Immunogenicity of pneumococcal conjugate vaccines in infants depends on several factors. Each individual serotype has by itself its own immune stimulating property which varies also depending on the protein carrier to which it is covalently bound (302) and the ratio between the carrier protein and the polysaccharides (303). Carrier priming may also affect the response (304). The age at which time the infant is immunized may play a major role (305) and also the number of primary vaccinations (217, 232, 306).

Antibody production, quality and function.

In all our studies we used the current consensus ELISA for pneumococcal polysaccharide antibody measurements (288, 307). The serum was not neutralized with serotype 22F as was later suggested based on a study showing that adsorption of antibodies to common epitopes in the sera increased the correlation between the IgG ELISA results and the OPA results (162). That study was done on adult sera before and after PPV23 vaccination but the same effect was not demonstrated in 7 month old infants who had previously been vaccinated with PCV. Further, when 22F absorption was used in sera from infants in the efficacy trial in the NCKP efficacy trial, it resulted only in minimal declines in pneumococcal antibodies in PCV7 immunized infants but significant declines in unimmunized controls. Serotype 22F absorption is therefore of more importance when measuring serum from individuals who have history of natural exposure or older individuals who have been vaccinated with PPV23 but has little effect when measuring serum from PCV vaccinated infants. With recalculation of the protective concentration after 22F absorption the level declined from 0.35 μg/ml to 0.32 μg/ml. A meta-analysis on pooled data form the NCKP trial, efficacy trial among American Indians and South African infants, derived a protective concentration of 0.35 μg/ml for anticapsular antibodies to the 7 serotypes in PCV7 (138) which is now recommended by WHO as a global basis to assess the immunogenicty of future PCVs as a critical goal for vaccine success (142). The concentration of 0.35 μg/ml was used in our last study

but prior to that we used the level of 0.15 µg/ml which was at the time our study was performed considered to reflect functional seroconversion measured by opsonophagocytosis and to discriminate vaccinated subjects from unvaccinated controls (308).

To evaluate the immunogenicity of a pneumococcal conjugate vaccine in infants we compared first the monovalent 6B-TT vaccine in two infant age groups and adults. We confirmed the immunogenicity of the vaccine in both infant groups but with higher IgG anti-6B responses observed after the two doses administered to the older group at 7 and 9 months compared with three doses given to infants at 3, 4 and 6 months (Paper I). A decrease was observed in 6B specific IgG after the first two doses of 6B-TT vaccine in the younger age group, which may partly be explained by interference by pre-existing maternal IgG antibodies at 3 months of age that was overcome first after the third dose. However, 6B is inherently a poorly immunogenic serotype, due to limited number of epitopes (309) which likely results in a low number of B-cells expressing the canonical idiotype in early infancy, which increases by repeated vaccinations. We observed similar kinetics after immunization with the 8-valent PCV8-DT or PCV8-TT vaccines, in which the IgG levels to serotypes 6B and 23F decreased at 4 months, while serotype 19F remained at the same level but subsequently the IgG to all the serotype increased significantly after the third dose at 6 months. Similar kinetics have been described in other studies in different populations (188, 189, 194, 241). Besides the younger age, the presence of the maternal antibodies may have influenced the antibody response at 3 months as the first dose at 7 months induced higher response. The association between high maternal pneumococcal antibodies and poor PCV response in the infants was demonstrated in an American Indian efficacy trial on PCV7 (225). In that study the infants were vaccinated with 3 doses of PCV7 before 6 months of age. It was observed that the increasing maternal pneumococcal antibody concentrations were associated with lower concentrations after the first dose of PCV7 for all serotypes except types 18C and 23F and a lower response to the third dose for all serotypes but 18C. This serotype specific interference by maternal antibodies on infant's antibody responses raises the question if it may in fact make the infant more susceptible to

infection by the same serotype and therefore possibly increase the risk of vaccine type disease in the first month after the initial vaccination (214).

Another explanation for higher response at 7 months is likely to be antigen priming by the TT carrier protein in the routine DwPT vaccinations that were given at 3, 4 and 6 months of age. The effect of carrier protein priming on antibody responses to *Haemophilus influenzae* type b conjugate vaccine was demonstrated in 1994 when the response of two groups of infants to *Haemophilus influenzae* type b polysaccharide-tetanus toxoid conjugate (PRP-T) or HbOC Hib conjugates were compared. One group received a DT vaccination at 1 month of age and subsequently injections of PRP-T or PRP oligosaccharide-cross-reactive mutant diphtheria toxin conjugate (HbOC) vaccines at 2, 4 and 6 months of age while the other group received only vaccinations at 2, 4 and 6 months with same vaccines. The DT primed infants responded to PRP-T with significantly higher antibody levels after all primary doses and demonstrated a significantly higher memory response to unconjugated PRP at 12 months of age (304). In another study the same carrier priming was observed when TT was administered prior to PRP-T vaccination but with no effect when administered concomitantly (310). The same study demonstrated suppressing interference by maternally derived TT antibodies on the infant's anti-PRP response. This has been confirmed in other studies showing enhancement of PRP-T response by carrier priming (311). Increased total dose of CRM_{197} carrier administered with Hib did not reduce response to CRM_{197} conjugate vaccines (312).

When compared with adult responses to the 6B-TT vaccine, both infants groups responded with predominantly IgG1, reaching adult levels, but few reached adult levels for IgG2 or IgA. This is in agreement with other studies on antibody isotype responses to same PCVs in children and adults (313,190) and to other PCV formulations (135, 314).

The primary mechanism of protection against pneumococcal infection is opsonophagocytosis mediated by antibodies to the PPS and complement, OPA may be the best *in vitro* correlate of protection (315, 138, 316). Opsonic activity of

antibodies elicited by 6B-TT in infants could be demonstrated after two or three injections with a marked increase after the booster injection at 18 months in most of the infants in group B vaccinated at 7 and 9 months but fewer in group A vaccinated at 3, 4 and 6 months (Trial I). Although in this first study few infant serum samples were analysed, the OPA correlated with total and IgG antibodies to serotype 6B PPS in both infant groups. It was concluded that the PCV formulation tested, 6B-TT, induced serotype specific antibodies in infants that had functional activity as demonstrated by OPA, indicating protective potential.

By comparing the 8-valent PCV8-DT and PCV8-TT vaccines, we investigated which protein carrier elicited better antibody response to each serotype in infants. Previously, a 4-valent diphtheria toxoid or tetanus protein carrier PCVs had been investigated in Finland and Israel assessing safety, immunogenicity and dose response. A dose effect was observed with the PCV8-DT conjugate vaccine with 3μg per serotype PPS being the optimal dose which was comparable to the response elicited by 1 μg PPS of the PCV8-TT (184, 207, 224). Based on the results from those studies the two 8-valent pneumococcal conjugate formulations compared were produced at the doses of 3 μg of each serotype in the PCV8-DT and 1 μg of each serotype in the PCV8-TT.

The specific antibodies elicited by the 8-valent PCV8-DT and PCV8-TT conjugate formulations, confirmed their immunogenicity when administered to Icelandic infants concurrently with the recommended infant vaccinations at that time, including the whole cell pertussis component in the DTP. The IgG responses were serotype specific and carrier dependent, with PCV8-DT inducing higher antibodies to serotypes 3, 9V and 18C, whereas PCV8-TT induced higher response to serotype 4 and similar antibody responses were induced by both formulations for serotypes 4, 14, 19F and 23F (Paper IV). Both trial vaccines elicited significant IgG responses to all serotypes in the vaccine with ≥ 88% of 7-month-old infants reaching IgG antibody concentrations ≥ 0.15 μg/mL and a significant proportion reaching 1 μg/mL. The high booster memory response, induced by native PPS in the PPV23 vaccine in both groups indicates successful priming by the PCV8-DT and PCV8-TT vaccines, although higher in the PCV8-TT primed group. This high booster

responses to the PPV23 are in agreement with previously reported responses to PPV23 booster in children primed with a PCV, independent of which PCV vaccine was used in infancy (153, 174, 181, 184, 208, 217, 222, 317), (241). The higher concentration of serotype specific antibodies induced by PCV priming and PPV23 booster in toddlers coincide with the peak incidence of nasopharyngeal carriage and transmission of pneumococci and therefore may seem to be a preferred schedule. In fact, PPV23 booster following priming with PCV and has been recommended to expand the serotype coverage in populations with high incidence of invasive pneumococcal disease (248). The polysaccharide booster in children primed with a PCV has, however, been questioned because of concerns of induction of hypo-responsiveness (246, 250) as will be discussed under the section on immunological memory. PPV23 booster has also been used to demonstrate existence of a memory following priming with various PCVs at various schedules (217, 315, 318). The PPV23 vaccine contains 25µg of each of the 23 serotypes and is licensed for use in adults and high-risk children, older than 2 years of age. The reason for higher antibody response elicited by PPV23 than PCV booster is not clear but may be due to higher dose of PPS (x 8 – 25 compared with PCV8-DT or PCV8-TT) that induce more naïve B-cells and/or memory B-cells. Similarly, the higher booster responses in the PCV8-TT primed children may indicate that more memory B-cells have been generated by priming with the TT conjugated PS than the DT conjugated PS reflecting more effective tetanus driven T-cell help. These results are consistent with immunogenicity of Hib conjugate vaccines containing TT-carrier compared to DT carrier (319).

After the PCV8-DT or PCV8-TT priming vaccinations the IgG1/IgG2 ratio did not discriminate between the vaccine formulations and the IgG subclass pattern after the booster vaccination with either the same PCV or the PPV23 was the same in both groups. However, the IgG1/IgG2 ratio was lower in both groups following the PPV23 booster, demonstrating a higher proportion of IgG2 antibodies following a PPS compared with protein-polysaccharide conjugate, indicating a higher priming or higher recall or memory responses in the group receiving the PCV booster. Other infant studies have shown an IgG1 dominating response to polysaccharides

conjugated to CRM_{197} (314), outer membrane protein of *Neisseria meningitidis* (PCV-OMP; Merck & Co.) (135) and to similar conjugates with Diphtheria toxoid and Tetanus protein carriers as in our study (320).

The protective efficacy of the antibodies as evaluated *in vitro* by opsonophagocytosis demonstrated that the antibodies induced by PCV8-DT and PCV8-TT are functional and thus have protective potentials. As the OPA correlated well with the IgG levels it was higher following vaccination with PCV8-TT than PCV8-DT for serotypes 6B. The correlation observed between IgG and OPA for all three serotypes evaluated was similar in both vaccine groups. Serotype 19F tended to induce higher IgG anti-19F than other serotypes but with similar OPA results. The OPA has been suggested to have a better predictive value for efficacy than antibody levels measured by ELISA and is therefore an important measurement when evaluating PCVs. Serotype 19F is very immunogenic in comparison with other serotypes but has around ten fold less function in OPA. (153, 159, 188, 321-323). OPA was also more likely to predict efficacy against otitis media than antibody levels (159) and reflects more precisely the risk of HIV-infected compared to healthy South-African infants (321).

When the quality was evaluated by measurement of avidity at 14 months the PCV booster tended to induce higher avidity antibodies than the PPV23 booster in both groups, significant for serotype 6B in the PCV8-DT group and for 23F in the PCV8-TT group. This difference in avidity between PCV and PPV23 booster-induced antibodies was also found in Finnish (324) and UK children (217).

In the *in vivo* mouse model we further demonstrated the protective capacity of the 6B specific antibodies by passively immunizing mice with post-vaccination toddler sera before challenging with either serotype 6B or 6A pneumococci. The protective level to either serotype in the mouse model corresponded to approximately 0.3 µg/mL which is close to the WHO recommended level of protection (142). A strong correlation was found between OPA and IgG levels and at low IgG anti-6A concentrations the OPA differed between protective and non-protective serum samples ($P<0.03$) at comparable IgG concentrations and avidity. The mouse model,

evaluating the infant sera after immunization with trial PCV8-TT, added an important proof of protective efficacy of the vaccine induced immunity *in vivo.* Similarily, we have shown good agreement between OPA and passive protection against 19F correlating with IgG anti-19F levels and some cross-protection was also observed against 19A(325)

Together with results from other trials, investigating 4 – 8-valent PCVs using DT or TT as carrier proteins (184, 207, 224), 11-valent PCV formulations were produced (PCV11-F3 formulation). By adding serotypes 1, 5 and 7F an additional coverage was gained; important for the developing countries where these serotypes cause significant morbidity and mortality (49, 326, 327). The aim with the 11-valent vaccine was to produce a vaccine with the best chance of having a single effective worldwide pneumococcal conjugate vaccine formulation.

If the T-cell help, provided by the carrier protein, is the limiting factor in the immune response to PCV, one way to improve the antibody response is to increase the T-cell help. In an attempt to improve the immunogenicity to four serotypes (6B, 9V, 18C and 23C) which induced the lowest primary antibody response in the preceding clinical trials, the PCV11-F3bis formulation was developed, containing both DT and TT carrier for these serotypes. Also, by using two carriers the antigenic load of any single carrier may be reduced in order to decrease the possibility of carrier-specific suppression (226). In the comparison of the PCV11-F3 and PCV11-F3bis we evaluated the advantage of using two carrier proteins for the four poorly immunogenic serotypes.

The primary objective was to measure the IgG antibody responses after the priming vaccinations at 3, 4 and 6 months and compare between the two groups receiving PCV11-F3 and PCV11-F3bis formulations. Both vaccines induced significant IgG responses to all serotypes, with highest responses to serotypes 3, 4, 7F and 19F and lowest to serotypes 6B and 23F. Compared with the PCV11-F3, the PCV11-F3bis formulation did not demonstrate an improved antibody response at 7 months. The conclusion drawn was that both vaccines were safe and immunogenic but combining two carriers for serotypes 6B, 9V, 18C and 23F did not enhance the antibody

responses. Although not significant, PCV11-F3 tended to induce higher immune responses to most serotypes after the primary series. OPA correlated with the IgG levels for five serotypes after the priming vaccinations, (in addition to 6B, 9V, 18C and 23F, OPA was done for serotype 19F which was identical in both formulations), and were comparable in both groups. However, the avidity was higher in the PCV11-F3bis group for the same serotypes, but also for 19F which was identical in both formulations.

These results indicate that the T-cell help is not the limiting factor in the primary antibody response to PCV. Rather, the paucity of serotype specific B-cells may be the explanation of low immunogenicity. This is in line with a study investigating the carrier-specific T-cell responses associated with serotype specific antibody responses to PCV7. In that study no differences were observed in T-cell help between the highly and poorly immunogenic serotypes (328).

In our study on PCV8-DT/TT, interference was demonstrated to the Hib conjugate which was conjugated to DT, causing lower anti-PRP responses in children immunized with the PCV8-DT compared to PCV8-TT. Potential interference induced by common protein epitopes that are administered simultaneously has previously been described for tetanus toxoid in Finnish and Israeli infants (226). In that study the IgG anti-Hib as well as anti-tetanus toxoid responses to a PRP-T conjugate, were lower in infants immunized concomitantly with 4-valent PCV-TT vaccine compared with those receiving PCV-DT conjugate. A dose response analysis showed also a inverse relationship between anti-Hib IgG and concentration of anti-TT IgG. The most widely used protein carriers in infant conjugate vaccines are proteins that are already well known vaccine antigens in other infant vaccines such as TT, DT and CRM197. It is therefore important to evaluate the carrier-induced effects on vaccine responses. In the Philippines, this relationship was also investigated in infants immunized at 6, 10 and 14 weeks with either an aluminum adjuvant containing eleven-valent PCV (PCV11-F3-alum; same as PCV11-F3 with aluminum adjuvant) or a meningococcal diphtheria-conjugate vaccine compared to a control group that received only DTwP/PRP-T. The GMCs and proportions of infants achieving protective antibody concentrations to DTwP and PRP-T vaccine

antigens were similar among the groups of infants vaccinated with PCV11-F3-alum or a meningococcal diphtheria-conjugate vaccine as compared to a control group that received only DTwP/PRP-T. Therefore no negative interference was observed in this study and the PCV11-F3-alum vaccine boosted the antibody response to the carrier proteins, measured at 18 weeks (230). The difference seen between Finnish or Israeli infants compared with those in the Philippines may possibly be due to differences in natural exposure and high prevalence of pneumococcal disease in the Philippines.

The co-administered vaccines also influence the antibody responses. This was demonstrated in Israel where DTaP compared to DTwP resulted in lower anti-pneumococcal antibody responses to the primary and booster vaccinations with the 11-valent PCV11-F3, to levels that were considered unacceptable (227). The carrier-specific epitope suppression described before (226), was in that study unmasked when the whole cell Pertussis component was replaced by the acellular Pertussis in the concomitant vaccine. The whole cell Pertussis provided therefore an adjuvant effect that was of critical importance for the immunogenicity of PCV11-F3. The adjuvant effect of the whole cell Pertussis has been described before for Hib conjugate vaccine (329). Since the acellular Pertussis vaccine has superior safety profile together with comparable immunogenicity to the whole cell Pertussis vaccine, the future vaccination against *Bordetella pertussis* will be in the form of acellular vaccine in the industrialized countries (330). The importance of coadministration of whole cell perutssis for the immunogenicity of PCV11-F3 conjugate vaccine lead to discontinuation of further development of the PCV11-F3 vaccine (227). However, the developing countries will most likely continue using wP, underlining the importance of these data on efficient induction of functional antibodies by the TT- and DT-conjugated PCVs (Trial IV and (230)). Although we observed interference between the pneumococcal and Hib-conjugates containing DT carrier[16] others have not shown interference between TT-conjugates co-administered

[16] Abstract: I Jonsdottir, S. Sigurdardottir, Th Gudnason, S Kjartansson, K Davidsdottir, KG Kristinsson, G Ingolfsdottir and O Leroy. Concomitant administration of octavalent pneumococcal

with wP (230, 331) and DT-conjugated Hib is not commonly used any more. In our study, PCV11-F3 and PCV11-F3 induced antibody concentration of $\geq$ 0.3 µg/mL in 90 – 100% vaccinees for all vaccine serotypes but 6B and 23F which were above 82% which fulfills the serological criteria developed by WHO for new PCVs (142)

Number of primary PCV doses in infancy

The currently licensed PCV, Prevnar/Prevenar is licensed to be administered in three primary doses with a toddler dose to boost the immunity. Many countries administer infant vaccinations in two primary doses with a booster dose between 12 and 18 months of age. In these countries, it would be of great value for parents as well as societies if PCV could be administrated with same schedule as other vaccines. In Trial V, we compared in a randomized design, the immunogenicity and safety of a 9-valent PCV and MnCC combination vaccine. The data from Trial V suggests that the 9vPnCMnCC administered either as a two-dose primary infant schedule (3 and 5 months of age) or as a three dose primary infant schedule (3, 4, and 5 months of age) followed by a toddler dose at 12 months of age, is safe and induces significant primary immune responses to both vaccination schedules, priming for similar memory responses at 12 months of age. Three primary doses provide higher IgG responses, but still very significant immune responses were observed to two-doses that may effectively protect against invasive pneumococcal diseases with less cost. The serotypes that demonstrate the poorest immunogenicity, serotype 6B and 23F are the two types that may need three primary doses to protect between the primary series and booster vaccination. However, the anti-6B-IgG increased from post primary to pre booster measurements, indicating natural boosting to successful priming. The clinical relevance of administering two but not three primary doses remains unknown in terms of potential impact on mucosal diseases.

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polysaccharide conjugate vaccine, PNC-D and *Haemophilus Influenzae* conjugate vaccine, PRP-D, sharing the carrier DT, may induce interference in infants. The 2nd ISPPD, Sun City, S-Africa, 19-23 March 2000.

Our study is so far, the only published randomized study that compares safety and immunogenicity of 2 vs. 3 primary PCV doses. One other randomized study is being conducted in Israel, comparing two vs. three primary doses of PCV in 668 recruited infants. In that study the investigators compare immunogenicity and nasopharyngeal carriage after immunizations with PCV7 at 2, 4 and 6 (*N*= 327), at 4 and 6 months (*n* = 171) or after no PCV (*n* = 170). In that study, the two-dose schedule induced significantly lower IgG GMC levels for serotypes 6B, 14, 18C and 23F at 7 months. The two-dose group tended to have higher acquisition of serotype 6B and 6A at 7 and 12 months compared with the three dose group which the authors relate to the lower immunogenicity of the two-dose schedule (332).

Two other studies have compared 2 vs. 3 primary doses with historical or nonrandomized collateral study controls. A Swedish study compared 3, 5 and 12 months schedule to historical controls vaccinated at 2, 4, 6 and 12 months demonstrating similarly lower primary response to serotypes 6B and 23F after two doses (232). The other study in the UK, compared data from one study immunizing at 2, 3, 4 and 12 months to another study immunizing at 2, 4 and 12 months. Similar antibody responses were found with avidity maturation, indicating memory development in both groups (217). When evaluated at 9 months in the Philippines, vaccination with three doses of 11-valent PCV8-DT/PCV8-TT conjugate at 6, 10 and 14 weeks of age gave comparable antibody levels at 9 months of age as a single vaccination at 18 weeks (333). In that study a boosting effect of a high rate of natural exposure has to be kept in mind. Due to vaccine shortage Prevnar was administered in other schedules than 3 + 1 in USA. According to the US Centres for Disease Control and Prevention's Active Bacterial Core surveillance on the invasive disease, the effectiveness of one or more doses against vaccine serotypes was 96% (95% C.I. 93–98) in healthy children and 81% (57–92) in those with coexisting disorders (334). In that study a booster gave a significantly better protection than three-dose infant schedule alone. In Italy, a two dose schedule with Prevnar had clear impact on respiratory tract infections indicating effectiveness of the two-dose schedule (335).

In Norway, PCV7 was introduced into infant immunization schedule at 3, 5 and 12 months in July 2006 with a catch up for all children born in 2006. A rapid decline in the incidence rate in IPD was observed among children less than 2 years of age already in 2007, from 47.1 to 13.7 cases/100.000 population. Incidence of NVTs remained stable and no vaccine failures were reported in fully vaccinated children (237). In UK, vaccination with Prevnar was launched in September 2006 in a 2-dose primary schedule at 2 and 4 months and a booster at 13 months of age. Already that same year a decrease in invasive VT pneumococcal disease could be observed. The trend for NVT may be similar as seen in USA with some increase in incidence (236).

Taken together, immunogenicity studies indicate that two doses may induce satisfactory immune responses to all serotypes but serotypes 6B and 23F. The booster responses indicate successful priming to these serotypes which may be protective, although during the time interval between priming and boosting the 2-dose groups may be at increased risk for disease caused by these two serotypes. These data are highly relevant for the decisionmaking for introduction of PCV in Iceland, where pediatric vaccines are routinely given at 3, 5 and 12 monhths (except for MnCC at 6 and 8 months). Decision on PCV implementation is expected in 2009. Clinical data from countries using 2 + 1 schedule do support the protective potential of this schedule. Protection on the mucosal level needs higher antibody concentrations and therefore the clinical effect of reduced dose schedule against middle ear infection and pneumonia between the priming and booster vaccination has to be observed.

Immunological memory

Short-term immunological memory

The most important goal of infant vaccinations is the induction of a long lasting protective immunity by the antigens administered. Immunological memory is therefore one of the keys to a successful vaccination. A PPV23 booster vaccination has been used to evaluate immunological memory induced by PCV administered in

infancy (336). At a young age when primary response to polysaccharide antigen is poor, a rapid increase of IgG to PPV23 booster, characterizes a memory response to the PPS antigen, induced by the PCV. Immunological memory was evaluated in this way in Trials II, III and V and immunological memory to all PCV serotypes was demonstrated. The results are in agreement with previous observations with PCV8-DT and PCV8-TT PCV vaccines (184) as well as CRM_{197} conjugated PPS (153, 174, 184, 208, 217, 222, 241, 317, 337). Short term immunological memory (defined in this thesis as less than one year after last vaccination) was evaluated by analyzing booster responses to a full dose of PPV23 in Trial II, III and V. In Trial II, 3 infant doses of 8-valent PCV8-DT or PCV8-TT resulted in significant memory responses at 13 months. Secondly in Trial III ,children who were vaccinated with one dose of 11-valent PCV11-F3bis vaccine at 17 months showed a ten fold increase in serotype specific IgG one week after receiving PPV23 at 27 months of age, with avidity maturation as well. This demonstrates that a single dose of the 11-valent pneumococcal conjugate vaccine induces B-cell memory in toddlers. Thirdly in Trial V, 12 month old children who were vaccinated in infancy with two or three doses of 9vPnCMnCC responded significantly to PPV23 booster in only 7 days. This is the first publication of IgG booster responses to PPV23 or PCV evaluated after seven days in a randomized trial to evaluate memory. Children previously vaccinated with 4 doses of PCV-PD in the Czech Republic and boosted with PPV23 in the fourth year of live showed significant IgG responses to all vaccine serotypes in 14 days (243) but no responses were detected 4 days after the 4^{th} dose at 12 months (222).

By evaluating the kinetics of IgG responses to PCV and PPV23, 1 and 4 weeks after booster, we demonstrated robust memory responses to all the PCV serotypes in children who had been primed with PCV in the three studies. In trial V the 9vPnCMnCC induced very brisk responses which exceeded the responses observed after the PPV23 booster. Peak levels induced by the PCV were measured only 7 days after booster vaccination and then declined significantly during next 3 weeks, whereas the IgG levels induced by the PPV23 continued to increase until 4 weeks after vaccination. This illustrates a difference in the nature of immune response to PCV booster compared to the native polysaccharide booster. The difference in the

kinetics of PCV and PPV23 responses may possibly be explained by the activation of both naïve and memory B cells by PCV, due to T cell help, but only memory cells by PPV23. In a recent study in adults PCV booster was shown to induce two antibody forming B-cell populations, but comparison with PPV23 booster was not done (338). When *SCID/SCID* mice transplanted with human B lymphocyte subsets were immunized with heat-inactivated *Streptococcus pneumoniae* or with a pneumococcal vaccine, both *S pneumoniae* and the PPS elicited IgM anti-polysaccharide and anti-protein antibodies from IgM memory B lymphocytes and an IgG anti-PPS and anti-protein response from switched memory B lymphocytes. In addition, IgG anti-PPS and anti-protein antibody responses were elicited from IgM-memory B cells, suggesting a versatile role of IgM-memory B-cells in antibody responses to T-independent and T-dependent antigens (125).

The rapid response to a challenge at one year of age in children who were vaccinated in infancy may be due to plasma cells that rapidly develop from memory B cells. The kinetics of MenC antibodies, plasma cells and memory B cell responses after booster vaccination with Meningococcus C conjugate was investigated in children at 12 months of age who had received primary vaccination at 2, 3 and 4 months of age (242). The frequency of MenC specific memory B cells had declined since post-primary vaccinations at 5 months and were first detected in peripheral blood 8 days after the booster. MenC specific plasma cells on the other hand were detected first after 4 days and peaked on day 6 with subsequent rapid decline to day 8-9 (242) as reflected by memory antibody responses.

Long-term immunological memory

Persistence of antibodies continuously produced by long-lived plasma cells and restimulation of long-lived memory cells are the keys to long term immunological memory against encapsulated bacteria such as pneumococcus. A combination of priming vaccination with PCV in infancy and PPV23 toddler booster dose has been considered in order to decrease cost and potentially to increase vaccine coverage (248). However, the polysaccharide booster in children who have been primed with a polysaccharide conjugate vaccine has been questioned because of concerns of

induction of hypo-responsiveness (reviewed in (250)). This was first observed following repeated doses of meningococcal group C polysaccharide (339). This was further demonstrated in 2001 in a follow-up study on 5-year-old children who had received a meningococcal A/C conjugate during infancy and a booster dose with same conjugate, meningococcal A/C polysaccharide vaccine or placebo (IPV). At five years of age, the children were challenged with 10 μg meningococcal A/C polysaccharides and their response evaluated 10 days later. The serogroup C bactericidal antibody titers after 10 days were significantly higher in the groups who at 2 years of age received the conjugate booster or placebo than those in the children who received the polysaccharide booster at two years of age or previously unvaccinated control group indicating that the polysaccharide challenge at 2 years of age interfered with a subsequent memory response (246, 250). Other studies have shown similar effect of meningococcal C polysaccharide in infancy (340). Decreased responses to PCV has been demonstrated in adult population who have been vaccinated with PPV23 vaccine, 1 year or 5 years before (341) compared with responses in PPV23-naïve population (342).

To investigate if PPV23 booster can deplete or exhaust B-cell memory generated by PCV vaccines in infancy and jeopardize the long-term protective immunity to pneumococci we evaluated the long-term memory in children at 7 years, who received the 8-valent PCV8-TT vaccine at 3, 4, 6 and either PCV8-TT or PPV23 booster at 13 months of age (Trial II). With almost 50% participation six years later, 32 children were brought back for evaluation of immunological memory and compared to 9 non-vaccinated children who served as controls. This additional study on long-term PPS specific immunological memory is underpowered as the power calculations aimed to answer the research question in the original study and only a limited number of participants could be invited to participate in the additonal study at 7 years of age. Six years after infant vaccination, levels of antibodies against two VT pneumococci were still higher than in unvaccinated children. This difference was not observed for the NVTs. The memory responses were demonstrated by IgG measurements 7 and 28 days after a fractional dose ($1/10^{th}$ of full dose) of PPV23. For the VTs (except for serotype 14) a trend for lower responses was observed in

children who received PPV23 booster compared to those who received PCV booster at 13 months of age. However, both groups showed significantly higher responses than previously unvaccinated controls indicating existence of memory to the VT whether boosted with PCV or PPV23 at 13 months. The pattern of the IgG responses was similar in both groups and resembled the PPV23 booster responses in Trial V with highest IgG levels measured 4 weeks after the PPV23 challenge. Although the IgG1/IgG2 ratio decreased from 14 months to 7 years in the children who were vaccinated with PCV in infancy they still had higher serotype specific IgG1/IgG2 ratio than unvaccinated children, explained by higher IgG1 levels but comparable IgG2. This reflects the persistence of IgG1-switched long-lived memory cells, possibly enhanced by natural exposure to pneumococci. Serotype specific memory responses at 7 years of age are predominantly of the IgG1 subclass for the immunogenic (19F) and prevalent (6B) VT, whereas IgG2 represents the majority of antibodies to serotype 23F and NVT. Serotype 23F mirrors therefore the poor immunogenicity in infancy but still the response induced by the PPV23 challenge at 7 years was beyond what was observed in unvaccinated children, indicating that immunological memory had been induced and was still present. The poor immunological memory to serotype 23F may possibly be explained by lack of natural exposure as only 5% of nasopharyngeal cultures obtained at the age 2 – 4 years were positive for serotype 23F in children participating in Trial IV (Figure 9).

The results of our studies showed that serotype-specific IgG levels induced by PCV in infancy wane rapidly, in agreement with what has been reported from other studies (210, 213), although at the time of booster at 18 months levels are significantly higher than in unvaccinated controls (241), but that may also depend on natural exposure.

This is the first study to demonstrate immunological memory six years after 3 doses of PCV in infancy and a PCV or PPV23 toddler dose. We concluded from this study that a booster vaccination with full dose PPV23 at 13 months does not deplete or inactivate memory B-cells in children who were immunized with protein conjugated pneumococcal vaccine in infancy. However, the nature of the booster response differs indicating differences in the B-cell memory pool. These results are in

agreement with results from a study on serogroup C meningococcal glycoconjugate vaccine, which was shown to induce persistent production of memory B cells in teenagers previously primed with the conjugate vaccine, whereas plain polysaccharide vaccine did not (343). Furthermore, a strong association was found between the level of MenC-specific Ab and the frequency of memory B cells measured at 1 month after 3-dose primary immunization with MenC conjugate vaccine in infancy, and the persistence of functional Ab at one year of age (242). Emerging data thus supports the use of the conjugate vaccine for sustained population protection against diseases caused by encapsulated bacteria.

In addition we have demonstrated that measuring rapid and strong antibody responses seven days after booster vaccination is useful to study the existence of immunological memory.

Effects at mucosal level

Pneumococcus is a normal part of the nasopharyngeal microflora. Nasopharynx is therefore important as a port of entry for invasive pneumococcal infections. A decrease in NP colonization is an important factor of PCV effectiveness in decreasing systemic as well as mucosal infections and transmission.

The effect of PCV7 on nasopharyngeal colonization has been published from four randomized trials, showing a decrease in nasopharyngeal carriage of VT pneumococci in vaccinated children in three, using quatrivalent DT or TT as carrier proteins (344) and seven- or nine-valent PCV-CRM_{197} (177, 345). In the fourth trial a reduction in nasopharyngeal VT pneumococci was observed in infants at 5 and 9 months of age previously vaccinated with PCV9 at 2, 3 and 4 months of age but not reaching significance compared to unvaccinated placebo group (346). In addition the PCV7 vaccination in USA has decreased IPD in the nonvaccinated population at all ages, demonstrating heard immunity by indirect protection (274, 275, 280, 334, 347).

In two of our randomized vaccine trials nasopharyngeal culturing was part of the trial design, either using DT or TT PCV. In Trial II, 8-valent PCV8-DT or PCV8-TT was administrated at 3, 4 and 6 months with the same PCV randomized against PPV23 at 13 months and in Trial IV the 11-valent mixed DT and TT carrier administrated at 3, 4, 6 and 13 months. The serotype prevalence in both of these trials was similar to what has been observed in other western European countries (50, 71, 82, 90) with serotype 6A, being the most prevalent one, followed by 6B, 19F, 23F, 14, 19A and 15 in decreasing order in Trial II performed in 1995-7. In Trial IV, two years later, 6A was even more prevalent but 6B had dropped to 4th place preceded by serotype 19F and 23F in 2nd and 3rd place as before. Each of serotypes 19A and 14 accounted for over 2% of positive cultures but the rest were under 2%. These numbers may however not reflect the natural prevalence of the VT pneumococci in Iceland as PCVs have been shown to decrease the nasopharyngeal colonization of the serotypes in the vaccinees. In Trial II, a decrease in VT was observed compared to a control group not vaccinated with PCV. The difference was mostly accounted for by serotypes 19F ($P<0.0001$) and 23F. Colonization by VRT was also decreased in the vaccinated group when compared to the control group indicating a cross-protection. This was mostly due to serotype 6A. This is in agreement with our results demonstrating the cross-protection of post-booster sera against serotype 6A in the *in vivo* mouse model (Paper II). A reduction in nasopharyngeal VT colonization in a day care setting as well as a reduction in antibiotic resistant serotypes has been reported following vaccination with nine-valent pneumococcal- CRM_{197} conjugate (348-350). The prevalence of 19A or 6A did not differ between PCV vaccinated and unvaccinated children at 24 months of age as has been observed following community-wide Prevnar vaccination in USA (284, 351). In trial II, some increase was observed in the prevalence of NVT pneumococci, only significant at 18 months of age. This was not observed in Trial IV when nasopharyngeal cultures were compared between vaccinees and unvaccinated playmates at the age 24 months of age. In that study an increase in VT pneumococci was noted at 18 months of age which may indicate some protection until that age which subsequently faded with time from vaccination, increased age

and community exposure. If the vaccines provided protection against mucosal colonization, the effect appeared to have disappeared by 24 months of age when colonization rates from both vaccine and non-vaccine types were comparable in both groups.

Replacement of VT with NVT pneumococci in nasopharyngeal carriage has been reported which may compensate for the decrease of VT leading to overall little effect of PCV at mucosal level (177, 352). Serotype replacement has also been observed in mucosal disease as reported in the AOM efficacy trial with PCV7 (193) and with the PCV-OMPC (181). Similarly, some increase in NVTs have been detected in culture positive middle ear effusion in the USA after PCV7 was introduced in 2000 (351).

Our results did not show increase in carriage of non-typable *Haemophilus influenzae* as has been described before (193, 353, 354) or *Moraxella catarrhalis*.

The efficacy of the PCV in decreasing IPD caused by VT is not debated (143, 272, 214, 279, 355-357). At the same time as VT pneumococcal disease has decreased significantly in all age groups resulting in decreased morbidity and mortality some increase has been reported in NVT diseases. According to a report on invasive cases through the Centers for Disease Control and Prevention's Active Bacterial Core surveillance IPD due to nonvaccine serotypes is increasing (358). Comparing rates of IPD caused by NVT in 1998-99 and 2004, the incidence rose in children less than 5 years ($p=0.01$) and also in adults above 65 years of age ($p=0.05$). Serotype 19A has become the predominant cause of IPD in children but serotypes 3, 15, 22F, and 33F were also observed. This trend has also been observed in a case control study in Spain (359).

In a recent retrospective study on the etiology of invasive bacterial infection in Icelandic children, from birth to 18 years of age, *Streptococcus pneumoniae* was the most commonly cultured bacteria. The most prevalent serotypes/groups were 14, 19 and 7 followed by 6B, 23, 6, 6A, 33, 18 (96). Another small study in Iceland looked at recurrent IPD in Iceland in 1975 – 2004 where 6B, 7F, 9V, 14, 16, 18C, 23F, 19F, 19A and 33F caused IPD (97). The serotype distribution in these two studies showed

serotype 7F being more common in IPD than in nasopharyngeal carriage in our vaccine studies. Although our trials were performed in the same time period a fluctuation has been observed in serotype distribution over time (87). A difference in serotypes colonizing the nasopharynx from those causing invasive disease has been described (86). In unvaccinated controls in Trial II, 49% of the total pneumococci cultured were of serotypes in the PCV7 vaccine and in the additional study to Trial IV 42% of controls carried PCV7 serotypes at 24 months. This is even lower than reported in the above study performed 1992 – 1999, showing that 51% of pneumococci cultured from children in daycare centers in Reykjavik were of PCV7 serotypes (87). The differences can partly be explained by the high prevalence of serotypes 6A and 7F in nasopharyngeal swabs from Icelandic children.

Nasopharyngeal colonization with *Streptococcus pneumoniae*, is a key step towards pneumococcal disease (113). Prevention of pneumococcal colonization is therefore a major issue in pneumococcal disease eradication, both to prevent invasive disease in individuals as well ad horizontal spread in communities. The PCV decreases vaccine type pneumococci on nasopharyngeal mucosa. However, the mechanisms induced by parenteral PCV vaccination reducing carriage of the VT pneumococcus at mucosal level are only partly understood.

We observed a relationship between frequency of nasopharyngeal colonization and serotype-specific IgG responses to the same serotype. This was first observed in Trial I and when the same observation was made in Trial II (data not included in this thesis) we specifically looked at this relationship in Trial IV in which more cultures were obtained allowing statistical comparison. Children who were found to be colonized with serotype 6B, 19F or 23F had lower IgG responses to the same serotype than children never colonized. This relationship was even stronger when the same serotype colonized more than once. This may indicate some mucosal protection of serotype-specific IgG. Similar findings were described in Israel where new acquisition of pneumococci of serotypes 14 and 19F inversely related to serum IgG one month after PCV vaccination. In that study an inverse relationship was also found between acquisition of serotype 6A to anti 6B IgG levels (148). Longitudinal analyses of nasopharyngeal carriage in Israeli toddlers also showed a lower risk of

colonization with serotypes 6A, 14 and 23F if previously exposed to the homologous serotype and a non-significant trend for serotypes 19A and 23A. No relationship was found for serotypes 6B, 19F and group 15 in that study. The authors concluded that their results implied that some serotypes generate anti-capsular antibodies that can reduce the risk of carriage in unimmunized toddlers (360). When analyzed in adult patients with COPD, the resistance to pneumococcal colonization was not related to levels of serum antibody concentrations to pneumococcal antigens (361). In a household study in UK, 121 families were followed with monthly nasopharyngeal swabs from all family members and blood samples form those who were above 18 years. The results showed that serotype-specific IgG increased significantly after carriage of serotypes 9V, 14, 18C, 19F and 23F and for serotype 14, high initial serum IgG was associated with reduced odds ratio of carriage. Similar but weaker association was found for pneumococcal protein antibodies (150).

We showed that IgG may play a role in mucosal protection. This is consistent with successful treatments of agammaglobulinemia patients with regular intravenous immunoglobulin administrations (362, 363) and by the success of Hib immunization to eradicate both colonization and disease (163, 301) as well as the heard effect from the PCV7 administered in infancy (364). The mechanism of protection on the mucosal level, induced by parenterally administered PCV is not clear.

It has been proposed before that at critical level, serotype specific IgG may be sufficient to protect against infectious disease by inactivating the inoculums of the pathogen (365). Colonizing pneumococci may induce inflammation leading to leakage of serum antibodies and efflux of phagocytes onto the mucosal surface.

Parenteral primary immunization of 2-year old toddlers with PCV led to increased frequency of PPS-specific IgA producing cells in the circulation one week later, indicating that the PCV stimulated IgA^+ memory cells previously induced by natural exposure on the mucosal site (366). In two studies on 7-valent CRM97 and OMPC PCVs, primed infants produced significant IgA responses in saliva but no memory responses were detected after PPV23 booster at 12 months (204, 367).

By using polymeric IgR knockout mice which lack the ability to actively secrete IgA onto the mucosal surface, the important role of secretory IgA in protection against pneumococcal colonization was shown when the IgA gene-deficient mice were not protected from colonization(368).

In an experimental model of human pneumococcal colonization, individuals experimentally colonized with serotype 23F developed serum IgG and secretory IgA responses to the pneumococcal PspA where as uncolonized individuals showed no change. The susceptibility to carriage did not correlate with the serum anti-23F IgG in the serum (369). Further experimental human colonization with serotype 6B and 23F showed significant rise in serum IgG to PspA and CbpA proteins indicating the role of pneumococcal proteins in the induction of mucosal immune responses (370).

Mouse data have shown that intranasal immunization with pneumococci confers CD4+ T-cell-dependent but antibody- and serotype-independent accelerated clearance of pneumococcal colonization (371) where IL-17 played a critical role (372). A successful priming and booster has been demonstrated with intranasal immunization with serotype 1 PCV with the adjuvant LT-K63, where the intranasal route was found to induce a higher memory response than the subcutaneous route in addition to eliciting salivary IgA response (373, 374).

Nasopharyngeal colonization precedes infection by the pneumococcus (113, 375) and the prevention of carriage by mucosal vaccination may therefore be the optimal goal for prevention of pneumococcal disease. Ideally should vaccine antigens provide a protection across all pneumococcal serotypes. The PCV vaccines are designed to fight invasive diseases in which it is highly effective. Some effect has been observed against otitis media mucosal disease but higher antibody levels, mucosal IgA and/or CD4 cells are needed for mucosal protection. This was also observed in a mouse study where one log higher antibody levels were required to protect against pneumonia compared to blood infection (376).

Serotype-specific hypo-responsiveness following nasopharyngeal colonization with pneumococci has recently been suggested in a study from Israel where infants who were colonized with vaccine serotypes before vaccination showed decreased primary

responses. The infants were vaccinated with PCV7 at 2, 4 (group 1) and 6 or 4 and 6 (group 2) months and cultures obtained from nasopharynx at 2 months or 2 and 4 months from group 1 and 2, respectively. The primary responses to serotypes 6B and 19F measured at 7 months were significantly lower in infants colonized with the same serotypes before vaccination in both groups. A similar trend was observed for serotype 23F (377), However, the memory responses at 12 months were not affected.

The effect of Prevnar immunizations in infancy on AOM was evaluated in two efficacy trials, showing 6% reduction in VT middle ear infection (193) and 7% reduction in AOM episodes (143). The third efficacy trial using 11-valent Protein-D conjugate (11 pneumococcal serotypes conjugated with *Haemophilus influenzae* – derived protein D) in the Czech Republic and Slovakia 33,6% efficacy in any specialist-confirmed clinical AOM episode (220). The pneumococcus along with nontypeable *Haemophilus influenzae* is the main bacterial cause of AOM (65, 66) and sinusitis (67) and the efficacy of PCV11-PD against nontypeable *Haemophilus influenzae* (source of carrier protein) AOM was 35.3% (220). In Alaskan Indians, The PCV7 protected against 64% of VT otitis media (271).

In the additional retrospective case control study to Trial IV comparing 64 vaccinees to 105 unvaccinated controls at 24 months of age, the parents of the PCV vaccinated children reported significantly less history of AOM than parents of the unvaccinated controls. Similarly, fewer reports of antibiotic prescriptions during the preceding 6 months and fewer tympanostomy tube insertions were reported by the parents of PCV vaccinated children. The study has some weaknesses; one being a retrospective study although only for 6 months; two, in spite of being selected by the daycare staff, the participation of parents of the control group may be biased, as parents of children with frequent AOM would be more willing to participate in a study investigating the effect of a vaccine that possibly might help against AOM. Our results, however, are in agreement of other studies investigating the effect of PCV on AOM.

CONCLUSIONS

All of the six pneumococcal conjugate vaccines investigated in the five clinical trials, proved to be safe when administered to infants in two or three doses with a toddler booster dose with either the same conjugate vaccine or the PPV23.

Each of the pneumococcal conjugate vaccines tested, induced significant serotype specific antibody responses after two or three primary vaccinations in infancy and in two trials immunological memory was demonstrated with robust booster responses to pneumococcal polysaccharide vaccine at 12-13 months of age. The functional capacity of the vaccine-induced antibodies was demonstrated by opsonophagocytosis and further by passive immunization in a mouse protection model *in vivo.*

By comparing DT and TT protein carriers for the same serotypes in two 8-valent PCVs our results supported the choice of an 11-valent mixed DT and TT carrier PCV with additional serotypes that are very important for the developing countries, namely serotypes 1 and 5, and also 7F which has been prevalent in Western Europe, including Iceland. This formulation was immunogenic in our studies when given with the Icelandic infant vaccinations at that time. It proved effective in an efficacy trial in the Philippines where it was also administered together with whole cell pertussis vaccine. It is a good candidate for infant vaccinations in the developing countries where whole cell pertussis is likely to be part of routine infant vaccinations, but the development was put on hold due to lack of immunogenicity when administered with acellular pertussis vaccine. Some interference of DT-conjugated PCV was observed with the DT conjugated Hib vaccine which is no longer in use, and this observation emphazises the importance of investigating the effect of new vaccines on vaccines given concomitantly. No benefit was shown from the mixed double carrier formulation for the poorly immunogenic serotypes, which is important information for future PCV developement. The results demonstrate safe and immunogenic vaccines that can protect infants against pneumococcal disease.

The number of primary PCV vaccinations during the first year of life is an important issue when PCV vaccination will be applied to the infant vaccination schedule in

Iceland, as well as in several other countries. Our results show lower antibody responses elicited by two primary doses compared to three, which is particularily significant for serotypes 6B, however with excellent memory responses at 12 months of age. The proportion of infants reaching the protective level for serotype 6B continued to increase until 12 months of age which indicates that this immune response may in fact be sufficient in Icelandic children where carriage of serogroup 6 is prevalent.

One very important result from these studies is the demonstration of immunological memory both at one year of age and six years after vaccinations. We also demonstrated that one PPV23 toddler dose does not destroy the memory generated by the conjugate vaccine in infancy, but it may change the nature of the immune response.

Pneumococcal conjugate vaccines tested in these trials can decrease the nasopharyngeal colonization of vaccine type pneumococci although that effect seems to wane with time from vaccination and increased community exposure. With community-wide infant vaccination we can decrease the incidence of pneumococcal diseases, morbidity and mortality. Furthermore, our results indicate that vaccination may reduce otitis media and antibiotic usage and therefore hopefully antibiotics resistance. By decreasing nasopharyngeal colonization the PCVs may reduce transmission and through heard immunity prevent pneumococcal diseases in unvaccinated vulnerable groups like the elderly.

Thus, implementation of routine infant vaccination with multivalent PCV as a preventive health measure should be seriously considered.

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APPENDICES

APPENDIX I: List of studies investigating safety, immunogenicity and efficacy of various PCVs.

N	Valency	PCV: serotypes and carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Main message	Ref.
1	1	6B-**TT**	Iceland	20: A 20: B	Open A: 2, 4, 6, 18 mo. B: 7, 9 and 18 mo.	Safety, IgM, IgG, IgA, IgG1/2, OPA	Safe, immunogenic, higher response in group B.	1997 and 1998 Sigurdardottir et al.
2	4	6B, 14, 19F, and 23F-**OMPC**	Finland	A: 62 infants B: 31 toddlers	Open: A: 2, 4, and 6 months B: 4, 6, and 14 Toddlers: 24 months or at 24 and 26 months	IgG	Significant IgG to 14 and 19F after the first dose, to 6B and 23F after the second dose. Booster response seen 14 months. Similar responses to 1 – 2 doses in toddlers as ininfants.	1995 Kayhthy H et al. J Inf Dis. 172(5);1273-1278
3	5	10 µg pr serotype 6B, 14, 18C, 19F and 23F- **CRM_{197}**	Finland	30	3 doses of PCV at 2, 4 and 6 months	Safety, IgG	Safe Produces significant IgG after 1 dose for 18C, after 2. or 3. doses for the other serotypes	1996 Åhman H et al. Ped Inf Dis J; 15(2):134-9
4	7	3.5 µg of type 6B, 2µg of 19F, 1.5 µg of 9V, and 1 µg of each of 4, 14, 18C and 23F - **OMPC**	USA	20: PCV at 2, 4, 6 and PPV23 at 12 – 15 mo. 13 PCV naïve: PPV23 at 12 – 15 mo.	Open, nonrandomized	Safety IgG	Highly immunogenic Memory responses to PPV booster but negligible responses to PPV in PCV naïve children.	1996 Anderson EL et al. Pediatrics;128(5I): 649-53
5	5	5µg pr serotype 6B, 14, 18, 19F and 23F- **CRM_{197}**	Gambia	30: 3 doses 30: 2 doses 30: Hib Placebo	3 doses 2, 3, 4 mo. or 2 doses 2, 4 mo. or Hib placebo.	Safety IgG	Safe Immunogenic Three primary doses gave higher levels than two	1996 Leach A Ped Inf Dis J; 15(4):333-9
6	4	3µg/serotype 6B, 14, 19F, 23F – **TT or DT**	Israel	25: PncD 25: PncT 25: Placebo	DBPCR PCV or placebo at 2, 4, 6 mo. All: PPV23 at 12 mo.	Safety, IgG	Safe, Both immunogenic Immunolgical memory	1997 Dagan R et al. Ped Inf Dis J.; 16(11):1053-9
7	5	10 µg of each 6B, 14, 18C, 19F and 23F oligosaccharides - **CRM_{197}**	USA	18 with HIV: PCV 33 without HIV: 17 PCV and 16 placebo	HIV positive: open HIV neg:: DBPCR PCV or Placebo in 2 doses with at least 2 months apart.	Safety IgG	Safe and immunogenic in both HIV infected and HIV free children.	1997 King Jr JC et al. Pediatrics;99(4): 575-80.

N	Valency	PCV: serotypes and carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Main message	Ref.
8	5	0,5 or 2 or 5 µg oligosaccharide (OS) or polysaccharide (PS) pr serotype 6B, 14, 18C, 19F and 23F- **CRM_{197}**	USA	400: 7 groups	DBPCR, 3doses of OS or PS PCV or placebo at 2, 4 and 6 months	Safety IgG	Safe All 5 serotypes immunogenic PS more immunogenic than OS, Dose response: 5 > 2 > 0.5 µg Improved Hib immunogenicity when given with Pn-CRM197	1997 Daum RS et al. J Inf Dis; 176(2):445-55
9	5	PPS following 5-valent PVC- **CRM197**	Gambia	84 from Follow-up study from (5)	Immunolgical memory response at 2 years in children who received PCV or Hib at 2, 3 and 4 or 2 and 4 mo.	IgG Avidity OPA	PCV primes for subsequent exposure to PPV	1997 Obaro SK Ped Inf Dis J; 16(12):1135-40
10	4	1, 3 or 10 µg/serotype 6B, 14, 19F, 23F – **DT**	Finland	25: 1 µg/serotype 25: 3 µg/serotype 25: 10µg/serotype 45: Placebo	DBPCR PCV or placebo at 2, 4 and 6 mo All: PPV23 at 14 mo.	IgG, effect of dose	Highest dose gave highest primary responses. Lowest dose gave highest booster responses	1998 Åhman H et al. Ped Inf Dis J; 17(3):211-6.
11	7	4, 6B, 9V, 14, 18C, 19F, 23F- **CRM_{197}** (PCV7)	USA	212 received either PCV or MnCC	DBPCR with either PCV or MnCC at 3, 4, 6 and 12 – 15 months	IgG	Safe All serotypes immunogenic and primend for a memory response at 12 months.	1998 Rennels et al. Pediatrics. Apr;101:604-11
12	7	4, 6B, 9V, 14, 18C, 19F, 23F- **CRM_{197}** (PCV7) or PPV23	USA	48 AOM prone and 64 AOM free children	DBR: PCV vs. PPV23.	Pre- and postimmunization IgG to serotypes 6B, 14, 19F, and 23F	Children including those with recurrent AOM, responded better to PCV than to PPV23. The difference in responses was primarily due to a better response to PCV in otitis-prone children.	1999 Barnett ED et al. Clin Inf Dis 29(1):191-2
13	4	6B, 14, 19F and 23F conjugated to **CRM_{197}** or **DT** or **TT** or **OMPC**	Finland	176 infants in consecutive, separate trials at with one of the four different PCVs	PCV at 2, 4 and 6 months of age. PPV23 booster to PCV- **CRM_{197}** (n=53) or **DT** (n=71) or **TT** (n=25) **OMPC** (no booster)	IgG Avidity	PncCRM or PncOMPC gave higher IgG to 14, 19F and 23F than infants given PncD or PncT Anti-6B was low throughout. No vaccine specific differences were found in avidity of anti-14 or -19F antibodies. Avidity to 6B and 23F differed significantly between the vaccine groups, PncCRM and PncT inducing antibodies of highest avidity.	1999 Anttila, M et al. Vaccine 17(15-16):1970-1977

N	Valency	PCV: serotypes and carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Main message	Ref.
14	7	4, 6B, 9V, 14, 18C, 19F, 23F- **CRM_{197}** (PCV7)	USA	302 healthy infants (Subset of ref (15)).	DBPCR with either PCV or MnCC at 3, 4, 6 and 12 – 15 months	Safety and IgG Effect from concurrent vaccines	Safe Immunogenic Somewhat lower antibody titers when given with DTaP and HbOC compared to separate vaccination, however, all antibody titres high in all subjects.	1999 Shinefield et al, J Ped Inf Dis 18(9):757-63.
15	9	2 µg of types 1, 4, 5, 9V, 14, 19F and 23F polysaccharides, 2 µg of type 18C oligosaccharide and 4 µg of type 6B polysaccharide – **CRM_{197}** (PCV9)	South-Africa	PCV9: 242 Controls: 239	DBPCR: PCV9 vs. true placebo at 6, 10, and 14 weeks	Safety Immunogenicity Impact on carriage	Safe. Significant antibody responses to all pneumococcal serotypes. *Haemophilus influenzae* type b and diphtheria antibodies were higher in children receiving PCV, compared with placebo recipients . VT carriage decreased in vaccinees at age 9 months, whereas carriage of NVTs increased.	1999 Mbelle et al. J Infect. Dis. 180:1171-6
16	4	1, 3 or 10 µg/serotype 6B, 14, 19F, 23F – **TT**	Finland	25: 1 µg/serotype 25: 3 µg/serotype 25: 10µg/serotype	DBPCR PCV 2, 4 and 6 mo All: PPV23 at 14 mo	Safety, IgG, effect of dose	One withdrawn due to adverse effect after 1st 10µg dose. No dose responses after primary vaccinations. The lowest primary dose gave highest booster.	1999 Åhman H et al. Vaccine; 17(20-21):2726-32.
17	7	3.5 µg of 6B, 2.5 µg 19F, 2 µg of 9V, and 1 µg 4, 14, 18C, and 23F – **OMPC**	USA	30 Alaska Native, 34 Apache and Navajo Indian and 32 non–Native American infants from KPSC.	Open Non randomized	Safety IgG	IgG responses after 3 primary doses similar in all 3 groups of children, except for serotypes 14 and 23F. Significant booster responses to all serotypes in all groups.	2000 Mienyk KM et al. Clinical Infectious Diseases.; 31(1):34-41.
18	7	4, 6B, 9V, 14, 18C, 19F, 23F- **CRM_{197}** (PCV7)	USA	47 children with sickle cell disease (SCD) and 14 children without SCD	Open lable SCD: **Schedule A**: PCV at 2, 4, and 6 mo. of age and PPV23 at 24 months: SCD, n=34; no SCD, n=11 or **schedule B:** PCV at 12 months and PPV23 at 24 months. SCD, n=13; no SCD, n=3.	Safety IgG	SCD respond to PCV7 at least as high as infants without SCD. Significant rises were seen in antibody concentrations to all 7VPnC serotypes after the PS-23 booster in children receiving schedule A or B.	2000 Obrien et al. Pediatrics. 106(5):965-72

N	Valency	PCV: serotypes a nd carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Outcome	Ref.
19	7	4, 6B, 9V, 14, 18C, 19F, 23F- **CRM_{197}** (PCV7)	USA	18927: ≥ 1 dose PCV 18941: ≥ 1 dose MnCC	DBPCR with either PCV or MnCC at 3, 4, 6 and 12 – 15 months	Safety, Immunogenicity Efficacy	Efficacy: IPD: 97.4%, No replacement AOM visits: 8.9% AOM episodes: 7.0% Frequent otitis: 9.3% Ventilatory tubes: 20.1% VT ear drainage: 66.7%	2000 Black et al Ped Inf D J 19:187-95
20	9	2 µg of types 1, 4, 5, 9V, 14, 19F and 23F polysaccharides, 2 µg of type 18C oligosaccharide and 4 µg of type 6B polysaccharide – **CRM_{197}** (PCV9)	Gambia	PCV9: 103 IPV: 104	Randomized: PCV vs. inactivated Polio (IPV) at 2, 3 and 4 months of age.	Safety, Immunogenicity Antigenic interaction with diphtheria, tetanus and pertussis vaccines	Safe. IgG to diphtheria and pertussis similar in both groups; However, IgG to tetanus toxoid was significantly lower in infants who received PCV.	2000 Obaro S et al. Ped Inf Dis J. 19:463-9.
21	7	4, 6B, 9V, 14, 18C, 19F, 23F- **CRM_{197}** (PCV7)	UK	368 infants randomized into three treatment groups for priming with PCV. PPV to all at 13 mo.	Randomized to receive at 2, 3 and 4 mo. 1) Controls: Routine vaccines (RV) only 2) Separate group: PCV7 with RV in a separate injection 3) Combined group: RV and PCV7 combined with HbOC	Reactogenicity Immunogenicity	Safe Significant IgG responses at 5 mo. but higher in the separate group than the combined group for five 7VPnC serotypes. Booster responses in both treatment groups at 13 mo.	2000 Choo et al. Ped Inf Dis J 19(9),pp 854-62
22	7	4, 6B, 9V, 14, 18C, 19F, 23F- **CRM_{197}** (PCV7)	Finland	60 healthy infants.	Immunized with PCV at 2, 4 and 6 mo. Booster with either PCV (N=30) or PPV23 (N=29) at 15 mo.	Immunogenicity in serum and saliva	PncCRM incuced both systemic and mucosal immune responses. At 7 months: 69 – 100% of children, depending on the serotype, had serum IgG > 1.0 µg/ml. Memory responses at 15 mo.	. 2001 Nurkka A et al. Ped Inf Dis J, 20(1);25-33
23	11	6B (10 µg), 3,14, and 18C (3 µg each) – DT and 1, 4, 5, 7F, 9V, 19F, and 23F (1µg each) – TT, with or without aluminum hydroxide adjuvant, **F3 or F3 alum.**	Finland and Israel	251 healthy infants.	Randomized, comparing PCV with or without aluminum adjuvant at 2, 4, 6, and 12 months of age	50 infants in each country: IgG concentration and avidity. 10 in each country IgG1/IgG2 subclasses.	Avidity increased between 7 and 12 months. Further increase after booster. The adjuvant improved avidity of anti-5 IgG. Mainly IgG1 subclass, although some IgG2 after boosting.	2001 Wuorimaa T et al J Infect Dis 184:1211-5

N	Valency	PCV: serotypes a nd carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Outcome	Ref.
24	8	3, 4, 6B, 9V, 14, 18C, 19F and 23F – **DT or TT**	Finland	50 healthy Finish infants.	Randomized trial with two PCVs: PncD or PncT at 2, 4, 6, and 15 months	Immunogenicity in serum and saliva. Safety	Safe Immunogenic to all VT. Response to PncD was higher for 3, 9V, 14 and 18C and to PncT for serotype 4. Salivary IgA and IgG anti-Pnc antibodies were measured for serotypes 4, 6B, 9V, 14, 18C, and 19F.	2001 Nurkka A et al. Vaccine 20:194-201
25	8	Serotypes 3, 4, 6B, 9V, 14, 18C, 19F and 23F conjugated with either **DT** (3µg pr serotype) or **TT** (1µg pr serotype)	Iceland	160 healthy infants	Randomized, open lable with PncD or PncT at 3, 4 and 6 months. Booster with either same conjugate or PPV23 at 13 mo.	Safety Immunogenicity	Significant IgG to all serotypes. The PncD gave higher primary responses to serotypes 3, 9V and 18C, whereas PncT gave better response to serotype 4.	2002 Sigurdardottir ST et al. Ped Inf Dis J 21(6):548-54.
26	11	6B (10 µg), 3,14, and 18C (3 µg each) – DT and 1, 4, 5, 7F, 9V, 19F, and 23F (1µg each) – TT, with aluminum hydroxide adjuvant, **F3 alum.**	Philippines	50 healthy infants	Open lable. PCV at 6, 10 and 14 weeks of age. PCV booster at 9 months.	Serotype specific IgG	High IgG against most pneumococcal serotypes after the first three doses of vaccine. The maternally derived antibodies did not decrease the response to the vaccine.	2002 Puumalainen T et al Ped. Inf. Dis. J. 21:309-14.
27	**11**	6B (10 µg), 3,14, and 18C (3 µg each) – DT and 1, 4, 5, 7F, 9V, 19F, and 23F (1µg each) – TT, with or without aluminum hydroxide adjuvant. **F3 or F3 alum.**	Finland, Israel Philippines	Philippino infants (n=51) Finnish infants (n=127) Israeli infants (n=124)	Randomized: F3 or F3 alum at 2, 4, 6 and 12 months	Serotype specific IgG	IgG to the TT conjugated polysaccharides were higher in the Filipino than in the Finnish or Israeli infants. F3 formulation induced lower GMCs than F3 alum.	2003 Puumalainen T et al. Ped Inf Dis J Vol 1, 22(2),141-149.
28	7	2µg of capsular PS of the serotypes 4, 9V, 14, 19F, and 23F, 4µg of type 6B PS, and 2µg of type 18C oligosaccharide – **CRM_{197}** (PCV7)	Finland	115 healthy infants from FinOM study 57: PCV7 58: Hepatitis B placebo.	Randomized: PCV vs. Hepatitis B at 2, 4, 6 and 12 months of age	IgA, IgA1, IgA2, IgG and sIg antibodies for serotypes 6B, 14, 19F and 23F in saliva at 7 and 13 months, and IgG and IgA also at 4–5 years of age.	The PncCRM induced both salivary anti-Pnc polysaccharide IgG and IgA. At 4–5 years IgA concentrations had increased in both groups and were similar. No evidence for the development of mucosal immunologic memory after vaccination with the PncCRM	2004 Nurkka A et al. Vaccine 23;298-304.

N	Valency	PCV: serotypes a nd carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Outcome	Ref.
29	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	Italy	46 pre-term (PT) G.a.:32–36 weeks 46 full-term (FT) G.a.:37-42 weeks	PCV7 at 3, 5 months (primary series) and at 11 months (booster) of age	Safety IgG	Comparable safety and immunogenicity in preterm and term infant after each of the three doses. Majority ≥0.35 μg/mL after 2nd dose.	2005 Esposito S et al. 23(14); 1703-1708
30	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	Sweden	101 healthy infants	Open, nonrandomized, multicenter study with PCV at 3, 5 and 12 months of age	IgG Tolerability	Two doses of PCV induced satisfactory antibody responses, with the exception of serotypes 6B and 23F. The third dose evoked strong responses for all serotypes, which suggests good immunologic priming with the primary series of 2 doses.	2005 Käyhty H et al. Ped Inf Dis J. 24(2):108-14.
31	9	1, 4, 5, 6B, 9V, 14, , 18C, 19F and 23F - **CRM197** (PCV9).	Gambia	8718: PCV 8719: True placebo	DBPCR; Three PCV or placebo at 6 – 51 week of age with intervals of at least 25 days between doses	Eficacy trial: 1) first episode of radiological pneumonia 2)clinical or severe clinical pneumonia 3) invasive pneumococcal disease 4) all-cause admission	Efficacy: Pneumonia: 7% (95% CI 1–12) VT IPD: 77% (51–90) All IPD: 50% (21–69) All-cause admissions 15% (7–21 Mortality 16% (3–28)	2005 Cutts FT et al Lancet 365: 1139–46
32	9	Combination 9-valent PCV and MenCC: 1, 4, 5, 6B, 9V, 14, 18C, 19F 23F, and MenC - **CRM197** (PCV9-MnCC)	UK	Pnc9-MenC: N=120 MnC-CRM197 placebo: N = 120	Randomized, controlled trial with PCV9-MnCC or MenCC at 3,4 and 5 months.	MnC immunogenicity measured by serum bactericidal titer (SBT) 1 month following the third dose. Safety	Pnc9-MenCC combination demonstrated reduced MenC immunogenicity compared with MnCC vaccine. The immunogenicity of Hib and DTwP was also diminished. Pnc9-MenC vaccine was safe and immunogenic for all pneumococcal serotypes.	2005. Buttery JP et al. JAMA 293:1751-1758
33	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7) or **OMPC**	Finland	166 infants om the FinOM trial	Randomized to PCV-CRM197 (n=56) vs. PCV-OMPC (n=46) vs Hep B placebo (n=58) at 2, 4, 6 and 12 months of age.	IgG antibody kinetics and Avidity	Both PCVs immunogenic, but different kinetics of antibody response; IgG anti-6B, -19F, and -23F declined faster after the 3rd and 4th doses in the PncCRM group than in the PncOMPC group. For both PCVs, the mean AI of anti-6B and -23F, but not of anti-19F, increased	2005 Ekström, N et al. Infect and Imm. 73(1), 369-377

N	Valency	PCV: serotypes a nd carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Outcome	Ref.
34	7	6B, (5 µg), 23F (3µg), 18C and 19F (2 µg each), 9V (1.5µg) and 4 and 14 (1 µg each) each conjugated to an outer membrane protein complex of N.meningitidis serogroup B, **OMPC.**	Finland	111 healthy infants from FinOM study 56: PCV-OMPC 55: Hepatitis B placebo.	Randomized: PCV vs. Hepatitis B at 2, 4, 6 months of age. At 12 months 49 received PCV and 7 PPV23.	IgA, IgA1, IgA2, IgG and sIg antibodies for serotypes 6B, 14, 19F and 23F in saliva at 7 and 13 months.	Salivary anti-Pnc IgG and IgA were detected more often in the PncOMPC than in the control group, significant only for 19F and 23F IgA concentrations at 7 months of age. At 13 months, antibody concentrations did not differ between PncOMPC and control groups.	2005, Nurkka A et al. BMC Infectious Diseases 5:41
35	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	Germany	127: PCV7 with DTPa-HBV-IPV/Hib. 126: Control with only DTPa-HBV-IPV/Hib	Immunized at 2, 3 and 4 months and 12-15 months of age	Safety Immunogenicity	Some differences in GMCs to the DTPa-HBV-IPV/Hib antigens after the primary series but GMCs for all antigens after the booster dose were similar in both groups, except for diphtheria which was significantly higher in the PCV7.	2006 Knuf M et al. Vaccine;24(22) 4727-4736
36	9	1, 4, 5, 6B, 9V, 14, , 18C, 19F and 23F - **CRM197** (PCV9).	UK	80 infants: 2-doses 80 infants: 3-doses	PCV9 in 2 or 3 doses at 2 and 4 or 2/3/4 mo. Booster with PCV or PPV23 at 12 months of age	Safety IgG GMC IgG Avidity	Post-primary IgG similar in 2- and 3-dose groups avidity maturation and booster responses similar PPV23 induced higher levels than PCV9	2006 Goldblatt D et al. Ped Inf Dis J 25(4);312-319
37	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	Canada	DTaP.IPV/Hib (PRP-T) and HB given at 2, 4, 6 months and randomly assigned (2:1) to receive PCV7 concurrently (n=246) or sequentially (at 3, 5, 7 months) (n=122).	Randomized, controlled trial with evaluator blinding.	Assessment of compatibility of concurrently administered PCV7, hepatitis B (HB) and DTaP.IPV/Hib vaccines.	Hib responses were increased (p = 0.008) and HB responses were reduced (p = 0.006) with concurrent dosing(same thight for HB). PCV7, DTaP.IPV/Hib and HB are compatible with concurrent, separate injections.	2006 Scheifele DW et al. Vaccine; 24(12):2057-64.
38	11	6B, 3,14, 18C,– **DT** and 1, 4, 5, 7F, 9V, 19F, 23F – **TT**, with aluminum hydroxide adjuvant, **F3 alum.**	Phillipines	180 infants	Randomized, controlled. All:DTwP/PRP-T, oral polio and hepatitis B vaccines at 6, 10, and 14 weeks of age and *N*= 57: 11PncTD at 6, 10 and 14 weeks. *N*=56: MenAC-TT *N*=55: 11PncTD at 18 weeks	DTwP and PRP-T immune responses IgG	Similar DTwP and PRP-T protection in all groups after primary immunization. 11PncTD induced a booster response D at 18 weeks in the control group. There was no negative interference from concomitant administration of conjugate vaccines.	2007 Ocampo AF et al Vaccine 25(4);605-611

N	Valency	PCV: serotypes and carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Outcome	Ref.
39	9	1, 4, 5, 6B, 9V, 14, , 18C, 19F and 23F - **CRM197** (PCV9).	South-Africa	HIV infected: PCV9: n=31 Placebo: n=49 HIV noninfected PCV9: n=98 Placebo: n=116	6,6 years follow up on S-Africa efficacy trial: mean age 5.3 y DBPC individual randomization	Efficacy IgG	HIV non-infected vaccinees had equal (serotypes 4, 6B, 14, 19F) or greater (serotypes 9V, 18C, 23F) proportions of serotype-specific antibody concentrations of ≥0.2 µg/ml to VT compared to HIV infected vaccinees at 5.3 years of age	2007 Madhi SA et al. Vaccine 25: 2451–2457
40	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7) or **OMPC**	Finland	166 infants om the FinOM trial	Randomized to PCV-CRM197 (n=56) vs. PCV-OMPC (n=46) vs Hep B placebo (n=58) at 2, 4, 6 At 12 months of age PCV or PPV23.	OPA, antibody concentration, and avidity for serotypes 6B, 19F, and 23F	OPA vaccine and serotype specific. PCV: positive OPA after the 4th dose, while none in the controls. Low IgG anti-6B but high anti-19F required for 59% killing. OPA correlated with antibody concentration, while avidity of antibodies did not.	2007 Ekström, N et al. Infect and Imm. 75(4):1794-1800
41	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	USA Alaska	315 PnCRM7 295 control subjects enrolled at < 7 months of age	PCV or control at 2, 4, 6 months of age	Immunogenicity	3-doses gave higher IgG than 2-doses for all serotypes but 14. Maternal antibody is associated with a reduced infant response to PnCRM7 but does not interfere with immune memory.	2007 O'Brien K et al. J Infect Dis;196:104–114
42	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	Bangladesh	214 infants	3 doses of a Hib conjugate vaccine with 3 doses of PNC7 vaccine at 4 weeks intervals beginning at 18 ± 1 weeks of age, with or without Zink supplementation.	Assessment the effect of zinc supplementation on IgG response to the PNC vaccine	Safe Immunogenic Zinc supplementation enhanced the immune response to only one of the serotypes (9V)	2007 Osendarp SJM et al. Vaccine:25(17); 3347-3354
43	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	UK	69 full term (FT) and 68 preterm infants (PT); (median gestational age 30 weeks)	PCV7 at 2, 3 and 4 months of age. PPV23 at 12 months of age	IgG, immunogenicity and memory induction	IgG lower in PT to six vaccine serotypes (ST) at 2 mo. and 5 mo. of age, to five ST at 12 mo. of age and to three ST at 13 mo. of age. At least 93% of both cohorts achieved IgG ≥0.35 µg/ml to all STs following booster vaccination.	2007 Ruggeberg JU et al. Vaccine:25(2); 264-271

N	Valency	PCV: serotypes and carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Outcome	Ref.
45	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	USA	Combined: 199 Separate: 188 Staggered: 188	Randomized, open. At 2, 4, and 6 months of age: 1) Combination: DTaP-HepB-IPV+PCV-7 +Hib or 2) Separate: DTaP+HepB+IPV+ PCV7+Hib or 3) Staggered: DTaP-HepB-IPV + Hib + PCV-7 administered 2 weeks later	Compare the immunogenicity of DTaP-HepB-IPV vaccine administered with PCV-7 and Hib vaccines with that of separately administered DTaP, HepB, IPV, Hib, and PCV-7 vaccines.	DTaP-HepB-IPV was highly immunogenic and well tolerated when coadministered with Hib and PCV-7 at 2, 4, and 6 months of age	2007 Pichichero, ME et al. J Pediatr 151(1);43-49
46	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	Sweden	101 healthy infants	open non-randomised multi-centre study: PCV7 at 3, 5 and 12 months. Duration of breast feeding calculated.	Duration of breastfeeding was calculated for days and correlated to rate of responders > 0,2 and 1,0 µg/mL.	Children exclusively breastfed 90 days or more might get a better serological protection against Hib and the pneumococcal serotypes 6B and 14 after vaccination, compared to children less breastfed.	2007 Silfverdal et al. Vaccine:8(9): 1497-1502
47	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	France	51 infants with sickle cell disease	PCV7 at 2, 3, and 4 months of age with a booster dose of PPV23 at 15 to 18 months of age	Safety IgG	After priming ≥95% of the subjects had antibody titers ≥0.35 µg/mL for the 7 serotypes. After boosting, geometric mean concentrations were high for all serotypes,	2007 Reinert, P. et al Ped Inf Dis J 26(12);1105-1109
48	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	Korea	202	PCV7 at 2, 4 and 6 months	Safety IgG	Safe After two and three PCV7 vaccinations, 63.0–98.0 and 97.2–100% of infants achieved an antibody level of ≥0.35µg/mL,	2007 Kim, Nam-Hee et al. Vaccine 25;7858–7865
49	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	China	800 infants	PCV7 at 3, 4, 5 months Group 1 (PCV7 ≥7 days before DTaP), or Group 2 (PCV7 with DTaP), or Group 3 (DTaP only)	Safety IgG	Safe For each vaccine serotype, at least 90% of subjects (Groups 1 and 2) had IgG concentrations >0.35 µg/mL after dose 3, except type 6B (Group 2) with 83.3%.	2008 Li, R.C et al. Vaccine 26(18), 2260-2269

N	Valency	PCV: serotypes and carrier protein	Country	Number of infants PCV /Contr	Trial design	Outcome measure	Outcome	Ref.
50	9	1, 4, 5, 6B, 9V, 14, 18C, 19F 23F, and MenC - **CRM197** (PCV9-MnCC)	Iceland	112 infants: 2 primary doses. 111 infants: 3 primary doses	Randomized study comparing 2 or 3 primary doses at 3 and 5 months or 3, 4 and 5 months.	Safety and immunogenicity	Three doses induced higher antibody GMCs at 6 months to seven of nine pneumococcal serotypes, most significant for 6B and 23F. A successful immunological priming demonstrated with PPV23 booster at 12 months.	2008, Sigurdardottir ST et al. Vaccine 26; 4178–4186
51	9	1, 4, 5, 6B, 9V, 14, , 18C, 19F and 23F **-CRM197** (PCV9).	Gambia	Subgroup of 212 Gambian children enrolled in a large vaccine efficacy trial	DBPCR with PCV7 vs. true placebo.	IgG Serotype-specific clinical vaccine efficacy from the main trial	% > 0.2, 0.35 and 1.0µg/ml, and the of anti-pneumococcal GMCs were higher for each serotype in vaccinees compared to placebo group. The estimated overall protective antibody level for all nine serotypes, based on the vaccine efficacy against vaccine-type invasive pneumococcal disease (IPD) of 77% (95% CI: 51, 90) observed in the trial, was 2.3µg/ml (95% CI: 1.0, 5.0)	2008 Saaka M. et al. Vaccine (26) 3719–3726
52	7	4, 6B, 9V, 14, 18C, 19F and 23F – **CRM_{197}** (PCV7)	Australia	53 cord bloods, 40 post-7PCV3 visits, 43 pre-23PPV visits, and 30 post-23PPV visits. Paired sera available for 16.	PCV7 at 2, 4 and 6 mo. and PPV23 at 18 mo.	IgG at birth and 1 mo. after primary vaccinations and befores and 1 mo. after PPV23 booster	After 3 doses of PCV7, GMCs were >1.95µg/ml and at least 89% of infants had IgG >0.35µg/ml to all 7PCV serotypes and to 23PPV given at 18 months of age 7PCV effectively primed for a booster response to 23PPV	2008 Leach A.J. et al. Vaccine 26 3885–3891
53	11	1 µg of each serotype; 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F conjugated individually to **protein D** (PDC)	Czech Republic	PDC coadministered with DTPa–HBV–IPV/Hib vaccine at 3, 4, 5 and 12-15 months of age.	DBPCR prospective efficacy study.	Safety after two years.	Safe .	2008 R. Prymula et al. / Vaccine 26;4563–4570

This table includes published safety, immunogenicity and efficacy studies of several PCV formulations containing five carrier proteins, CRM_{197}, OMPC, DT, TT or Protein D in different populations, listed in chronological order. This is not a complete summary of all publications reporting results of these PCVs.

APPENDIX II: A List of abstracts with results presented in the thesis

Safety p. 51:

2. Abstract: **S Sigurdardottir**, Þ Gudnason, KG Kristinsson, S Kjartansson, K Davídsdóttir, G Ingólfsdóttir, M Yaich, O Leroy, I Jónsdóttir. Safety And Immunogenicity Of Two Different Formulations Of 11-Valent Pneumococcal Polysaccharide Conjugate Vaccines, F3 And F3bis In Healthy Icelandic Infants. The 2nd ISPPD, Sun City, S-Africa, 19-23 March 2000

Comparison of Diphtheria toxoid and Tetanus protein as carriers in an 8-valent pneumococcal conjugate vaccine p. 62

3. Abstract: I Jonsdottir, **S. Sigurdardottir**, Th Gudnason, S Kjartansson, K Davidsdottir, KG Kristinsson, G Ingolfsdottir and O Leroy. Concomitant administration of octavalent pneumococcal polysaccharide conjugate vaccine, PNC-D and *Haemophilus Influenzae* conjugate vaccine, PRP-D, sharing the carrier DT, may induce interference in infants. The 2nd ISPPD, Sun City, S-Africa, 19-23 March 2000.

Protective capacities - Opsonophagocytosis, p. 63

4. Abstracts: I. Jonsdottir, **STh Sigurdardottir**, G Vidarsson, G Ingolfsdottir, Th Gudnason, K Davidsdottir, S Kjartansson, KG Kristinsson og O Leroy. Pneumococcal conjugate vaccines elicit functional antibodies in infants. 27th Scandinavian Society for Immunology Meeting, Turku, Finland, May 24 – 27, 1996. Scand J. Immunol 1996, 43:710
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In discussion

16. Same as in 2.