Man versus Microbe---who gets pneumococcal disease and why?

Invited Lecture Abstracts

ISPPD-0536
Man versus Microbe---who gets pneumococcal disease and why?

THE INFLUENCE OF HUMAN FACTORS: INCREASING AGE

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Advanced age is associated with enhanced susceptibility to severe invasive pneumococcal disease. This is exemplified by the fact that respiratory tract infections are the 4th leading cause of death for the elderly worldwide. One reason for this is the chronic low-grade inflammation that develops with advanced age (i.e. inflamm-aging). Inflamm-aging results in the expression of host-proteins co-opted by *Streptococcus pneumoniae* for lung cell adhesion and tissue invasion. Most recently, we have determined that inflamm-aging also contributes to alveolar macrophage dysfunction; specifically the inability of aged macrophages to respond to bacteria with a robust pro-inflammatory cytokines response. We tested the hypothesis that chronic inflamm-aging induces production of A20, a homeostatic suppressor of the NFκB and MAPK signaling cascades, and this is responsible for the muted cytokine response by alveolar macrophages. Comparison of tissues from young, mature, and aged C57BL/6 mice indicated that A20 was strongly elevated in lungs and alveolar macrophages from aged mice. Following co-incubation of macrophages with *S. pneumoniae*, TRAF6 polyubiquitination, the target of A20, was diminished in alveolar macrophages isolated from aged versus young mice. A20 production was inducible in macrophages by overnight incubation with TNFα but not IL-6; with TNFα treated macrophages having poor IL-6 production following their exposure to *S. pneumoniae*. Retrovirus-induced expression of A20 in macrophages resulted in their diminished production of IL-6 following their exposure to *S. pneumoniae* but had no effect on levels of phagocytosis. We conclude that elevated A20 due to TNFα contributes to poor macrophage function in the elderly with pneumonia.

No conflict of interest

Oral Poster Abstracts

ISPPD-0223
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RESPIRATORY MICROBIOTA DYNAMICS FOLLOWING STREPTOCOCCUS PNEUMONIAE ACQUISITION IN YOUNG AND ELDERLY MICE

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Background and Aims: The upper respiratory tract (URT) is a distinct microbial niche of low-density bacterial communities and also a portal of entry for many potential pathogens, including *Streptococcus pneumoniae*. Thus far, animal models have been used to study dynamics of and interactions between limited numbers of different species in the URT.
Methods: We applied a deep sequencing approach to explore, for the first time, the impact of *S. pneumoniae* acquisition on URT microbiota in a mouse model, and potential age-dependent effects. Young-adult and elderly mice were inoculated intranasally with *S. pneumoniae*, and nasal lavages were collected for up to 28 days post-colonization. Bacterial DNA extracted from lavages was subjected to barcoded pyrosequencing of the V5-V7 hypervariable region of the small-subunit ribosomal RNA gene.
Results: We observed highly diverse microbial profiles with the overall presence of 15 phyla and approximately 645 OTUs. We noted differences in microbiota composition between young and elderly mice, with a significantly higher abundance of Bacteroidetes in the young mice. The introduction of *S. pneumoniae* into the URT led to a temporary dominance of pneumococci in the microbiota of all mice, accompanied by a significant decrease in microbial diversity. As mice gradually cleared colonization, diversity returned to baseline levels. Diversification was accompanied by an early expansion of Bacteroidetes, *Staphylococcus spp*. and Lachnospiraceae. Moreover, Bacteroidetes expansion was significantly higher in young-adult compared to elderly mice.
Conclusion: We observed differences in URT microbiota composition between naïve young-adult and elderly mice, which were associated with differences in pneumococcal clearance in time.

No conflict of interest

ISPPD-0066
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INTERLEUKIN-7-DEPENDENT B LYMPHOCYTES ARE REQUIRED FOR THE ANTI-PNEUMOCOCCAL POLYSACCHARIDE RESPONSE AND PROTECTIVE IMMUNITY TO STREPTOCOCCUS PNEUMONIAE

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Unlike human adults or adult mice, young children or young mice respond poorly to pneumococcal polysaccharides (PPS). In mice, B1b lymphocytes are the major responders to a variety of bacterial polysaccharides including PPS. Despite having B1b cells, young mice are impaired in responding to PPS, suggesting that B cells in the young are distinct from those in adults. Since B lymphopoeisis early in life is Interleukin-7 (IL-7)-independent, while in adults it is IL-7-dependent, we hypothesize that B cells developed in the presence of IL-7 are required for generating anti-PPS antibody responses. In support of this, we found that despite having B1b cells, young wildtype and adult mice deficient either in IL-7 or IL-7Rα are severely impaired in responding to Pneumovax®23 vaccine, and do not survive *S. pneumoniae* challenge. Furthermore, we found that transgenic expression of IL-7 promotes the anti-PPS response in young and confers protective immunity. To translate these findings to human infants we have utilized neonatal NOD/SCID/γc^{null} mice engrafted with human umbilical cord blood $CD34^+$ hematopoietic stem cells to create a "Human Immune System" mouse (HISmouse) model. We have found that these HISmice generate several B cell subsets including B1 and the majority of them exhibit an immature phenotype. Moreover, just as young children, HISmice responded poorly to PPS. IL-7 is produced mainly by non-hematopoietic stromal cells, and unlike the human IL-7, the murine IL-7 is poor stimulator of human B lymphocyte development. These data indicate that IL-7-dependent B cells play a crucial role in generating anti-PPS responses.

No conflict of interest

ISPPD-0420
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PHOSPHOINOSITIDE 3-KINASE DELTA GENE MUTATION PREDISPOSES TO RESPIRATORY INFECTION AND AIRWAY DAMAGE

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Background and Aims: We report the discovery of a novel cause of primary immunodeficiency, Activated PI3 Kinase Delta Syndrome, or APDS, characterised by susceptibility to *Streptococcus pneumoniae*.

Methods: Exome sequencing of a cohort of patients with primary immunodeficiency has identified a recurrent missense mutation in the gene encoding the p110delta isoform of PI3 kinase in a number of families that was absent from over 3,000 healthy genomes – p110delta.E1021K. We show that this mutation arises spontaneously and is inherited in an autosomal dominant fashion, causing activation of this kinase leading to increased generation of the second messenger PIP3.

Results: APDS patients are clinically characterised by extreme susceptibility to infection, most notably with encapsulated organisms including *S. pneumoniae* and *Haemophilus influenzae*. They suffer from recurrent sino-oto-pulmonary disease culminating in progressive lung damage more severe than that seen in cystic fibrosis sufferers. Subjects often die before the age of 40. They have increased circulating IgM and reduced IgG2, and a marked expansion of T cells with an exhausted phenotype. Their T cells are susceptible to activation induced cell death and have disordered cytokine secretion in response to stimulation.

Conclusion: APDS is a novel cause of primary immunodeficiency that is typified by recurrent infection with *S. pneumoniae*. Discovery of this disease has coincided with inhibitors of p110delta being tested in phase III clinical trials for leukaemia. Mutant p110delta remains fully amenable to inhibition with small molecule inhibitors, thus these compounds may represent a viable treatment strategy for individuals with APDS.

No conflict of interest

ISPPD-0302
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MATHEMATICALLY MODELLING OF EARLY INTERACTIONS OF *STREPTOCOCCUS PNEUMONIAE* WITH THE HOST DURING THE DEVELOPMENT OF PNEUMONIA

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Background and Aims: Alveolar macrophages have an important role for controlling invading *Streptococcus pneumoniae*, thereby dictating whether pneumonia develops. However alveolar macrophages are only one component of lung host defences, and there are few data on bacterial factors that may affect early lung clearance. We have now investigated the effects on early lung clearance of alveolar macrophage dependent and independent immunity, *S. pneumoniae* replication rate, and the capsule.

Methods: Mice with or without alveolar macrophages depletion were inoculated with 5 x 10^6 colony forming units (CFU) of TIGR4 wild-type, non-replicating (ΔpabB) and unencapsulated *S. pneumoniae*. Bronchoalveolar lavage fluid (BAL) were plated to obtain bacterial CFU up to 4 hours post-inoculation. These data were analysed by mathematical modelling to calculate t1/2s for alveolar macrophage dependent and independent clearance, and the *S. pneumoniae* replication rate.

Results: Log_{10} CFU bacterial clearance was linear for the first 2 hours post-infection. Calculated half-lives for alveolar macrophage dependent and independent bacterial clearance and the *S. pneumoniae* replication rate are shown in the table:

	TIGR4 encapsulated	P1672 unencapsulated
Bacterial doubling time (mins)	16	20
Half-life of AM-dependent clearance (mins)	135	31
Half-life of AM-independent clearance (mins)	24	14

Conclusion: The results show (a) surprisingly rapid *S. pneumoniae* replication during early lung infection; (b) that the capsule inhibits both alveolar macrophage dependent and independent clearance; and (c) for clearance of encapsulated bacteria alveolar macrophage independent dominated alveolar-dependent clearance during early lung infection. These data will help explain why there is an increased susceptibility to pneumonia for some at risk patient populations.

No conflict of interest

ISPPD-0118
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RISK OF INVASIVE PNEUMOCOCCAL INFECTION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: A NATIONWIDE DANISH COHORT STUDY

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Background and Aims: Inflammatory bowel diseases (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC) are a result of an inappropriate immune response. Therefore, the main purpose of the medical treatment is to moderate the immune response thus reducing disease activity, leading to a theoretically increased risk of invasive pneumococcal infection (IPI). The objective of this study was to examine the impact of IBD on the risk of IPI.

Methods: Patients diagnosed with IBD from 1977 to 2013 were identified from the Danish National Patient Register. For each IBD patient, 20 individuals matched according to sex, age, and municipalities were selected from the Danish Civil Registration System. The IBD and control group data were linked with IPI data from the national laboratory surveillance.

Using Cox regression with time since onset of IBD/date of matching as underlying time axis we calculated hazard rate ratios (HRRs) for IPI after IBD.

Results: Among 83,358 IBD cases we found 316 IPI cases giving an incidence of 38 per 10,000, whereas the controls had an incidence of 26 per 10,000. The HRRs for CD and UC within the first 6 months after IBD diagnosis were high (>3) and then decreased to a constant level which for CD was significantly higher (approximately twofold) than for the controls and for UC non-significantly just above 1.

Conclusion: We found an increased risk of IPI infections among patients with IBD, which was most pronounced in the first years after diagnosis but remained increased over time, especially in CD.

No conflict of interest

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ASSOCIATIONS BETWEEN INDIVIDUAL CONTACT PATTERNS, CROWDING FACTORS AND PNEUMOCOCCAL ANTIBODY CONCENTRATIONS DURING THE PRE-VACCINE ERA IN THE NETHERLANDS

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Background and Aims: Young children and elderly are particularly vulnerable for invasive pneumococcal diseases. The aim of this study was to determine whether crowding factors and individual contact patterns are associated with pneumococcal antibody concentrations in young children and elderly.

Methods: In 2006-2007 a population-based serum bank was set up in the Netherlands for individuals aged 0 to 79 years of age to obtain insight into seroepidemiology, in particular, of vaccine preventable diseases. Blood samples were analyzed using fluorescent-bead based multiplex assay to obtain serotype-specific pneumococcal IgG concentrations. Associations between crowding factors, individual contact patterns and pneumococcal IgG antibody concentrations of 13 serotypes were analyzed with generalized estimating equations (GEE) for children aged 2 months to 3 years (n = 642) and elderly of ≥65 years (n = 1174).

Results: Young children had significantly higher pneumococcal antibody concentrations in the crude model when they attended a day care center or had contact with other young children. The adjusted model showed higher antibody concentrations when children lived in households consisting of more than 4 persons. Elderly had significantly higher concentrations of antibodies in the crude model when they had contact with 5-19 year olds. In the adjusted model, elderly who reported contact with 20-59 year olds, had lower antibody concentrations.

Conclusion: Individual contact patterns and crowding factors are associated with pneumococcal antibody concentrations in young children and elderly in the Netherlands. To our knowledge, this is the first time that GEE analysis is used to analyze data of different pneumococcal serotypes.

No conflict of interest

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ATF3 PROVIDES RESISTANCE TO STREPTOCOCCUS PNEUMONIAE INFECTION VIA CYTOKINE UPREGULATION

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Activating transcription factor-3 (ATF3) plays a crucial role in regulation of innate immunity. However, how ATF3 regulates innate immunity against Gram-positive bacterial infection remains unknown. Here, we investigated role of ATF3 for host defense upon *Streptococcus pneumoniae* infection. Pneumococcal infection induced ATF3 significantly high in various cell lines *in vitro* and many organs *in vivo*. Surprisingly, pneumolysin (PLY) induced ATF3 via Toll-like receptor 4 (TLR4) signaling. Moreover, pneumococcal infection induced ATF3 resulted in positive stimulation of production cytokines (TNF-α, IL-1β, and IFN-γ). In pneumonia model infection, wild type mice are more resistant than the ATF3 knock-out mice. Thus, ATF3 induced-cytokines might protect the host from pneumococcal protection. Taken together, ATF3 regulates innate immunity positively upon pneumococcus infection via TNF-α, IL-1β, and IFN-γ secretion resulting in clearance of pneumococci.

No conflict of interest

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IL-17 HAS BENEFICIAL OR DETRIMENTAL EFFECTS ON OUTCOME IN PNEUMOCOCCAL PNEUMONIA THAT ARE STRAIN DEPENDENT

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Introduction: IL-17 is a cytokine that promotes the release of anti-microbial factors and neutrophil recruitment. We investigated the role of IL-17 in a murine model of pneumococcal pneumonia.

Methods: IL-17RA-/- or wild-type mice were infected via the intra-nasal route with either TIGR4 (serotype 4) or strain SRL1 (serotype 3).

Results: TIGR4 produced high-level bacteremia in infected animals whereas the serotype 3 strain SRL1 caused dense consolidative pneumonia and pleural infection, but only invaded the blood stream late in the course of infection. IL-17A was present in the lungs of mice infected with both strains of pneumococci within 6 hours of infection. IL-17RA-/- mice had decreased expression of IL-17A target genes and delayed recruitment of neutrophils to blood and lung following infection with TIGR4 and SRL1. In TIGR4 infection, IL-17RA-/- mice had increased bacteremia and increased mortality compared to wild-type mice. In contrast, IL-17RA-/- mice had decreased mortality compared to

wild-type when infected with SRL1. *In vitro* studies confirmed that neutrophils effectively phagocytosed and killed TIGR4 whereas SRL1 was highly resistant to phagocytosis. When mice were treated with a neutrophil depleting monoclonal antibody, neutrophil recruitment was delayed and a similar trend in mortality was seen.

Conclusion: IL-17 signaling has a variable effect on outcome in this model, which depends on pneumococcal strain. IL-17 recruitment of neutrophils is crucial to host defence against an invasive serotype 4 pneumococcal strain but worsens outcome in infection with a heavily encapsulated serotype 3 organism. Thus, Th17 immunity may not always be protective in pneumococcal infection.

No conflict of interest

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DOES EXCESSIVE OXIDATIVE STRESS DIRECTLY CONTRIBUTE TO INCREASED SUSCEPTIBILITY TO PNEUMOCOCCAL INFECTIONS?

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Background and Aims: Excessive oxidative stress is a pathological feature of chronic lung diseases including COPD. As a large proportion of COPD patients are chronically colonized with respiratory pathogens, including *Streptococcus pneumoniae* (the pneumococcus), our aim is to identify the cellular and molecular mechanisms that underlie susceptibility to pneumococcal infections in the airways under high oxidant burden.
Methods: An experimental mouse model was used in which C57BL/6 mice were intranasally infected with *Stretococcus pneumoniae* EF3030 and coinfected with influenza A virus (IAV) to trigger pneumococcal disease. To model the increased levels of extracellular superoxide radicals detected in COPD airways, mice lacking SOD3 (superoxide dismutase 3) were used.
Results: Infection of SOD3-/- mice with pneumococci resulted in substantial weight loss (>15%) over a seven day period compared with infected wildtype mice. The pneumococcal load in the lung and nasal tissues was also increased in SOD3-/- mice compared to wildtype mice. Co-infection wth IAV resulted in dramatically increased pneumococcal numbers in the lungs and the nose, with more pneumococci in co-infected SOD3-/- mice compared with co-infected wildtype mice. Flow cytometry analysis of bronchoalveolar lavagefound that co-infected SOD3-/- mice had two-fold higher numbers of recruited inflammatory cells, in particular neutrophils.
SOD3-/- mice show increased susceptibility to infection with pneumococci, suggesting that pathogenesis of *S. pneumoniae* may be altered under oxidative stress. We are currently exploring differences in pneumococcal and host gene expression that may explain these changes insusceptibility to pneumococcal infection.

No conflict of interest

Poster Abstracts

ISPPD-0209
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STREPTOCOCCUS PNEUMONIAE INTERACTION WITH BRAIN-DERIVED GLIOBLATOMA CELLS AND ALTERATION IN FUNCTIONAL STATE OF THE CELLS

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Background and aims: *Streptococcus pneumoniae* causes meningitis with high mortality rate and 50% of survivors present sequelae including learning impairment, deafness, mental retardation and hydrocephalus. We have previously identified several putative bacterial adhesin and their respective receptors on epithelial cells in the host that are proteins known to function in brain development or affect neural cell function. We hypothesize that the very same adhesins are involved in the interaction of the bacteria with neural cells and may affect neural cell function and survival.

Method: To study alterations in the functional state of the U251 cells following infection with the pneumococci we tested DNA Topoisomerase I (Topo I) activity. Topo I is an essential nuclear enzyme that participates in all DNA transaction processes and is important for gene expression.

Results: We demonstrate a significant reduction in pneumococcal adhesion to glioblastoma cells (U251) in the presence of the recombinant adhesins (rPtsA and rGtS). Moreover, bacteria lacking the putative adhesins demonstrate reduced adhesion to U251 cells in comparison to the wild type bacteria. Infection of U251 with *S. pneumonia* inhibited Topo I activity significantly, albeit the mutated bacteria inhibited Topo I activity to a significantly lesser extent then the wild type bacterial strain.

Conclusion: The bacterial adhesins previously found to mediate bacterial epithelial cells interactions were currently found to mediate adhesion of pneumococci to glial cells and alter Topo I activity in these cells. Thus, these adhesins may be involved in pneumococcal meningitis development.

No conflict of interest

ISPPD-0028
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IMPAIRED TACI EXPRESSION IS RESPONSIBLE FOR THE UNRESPONSIVENESS OF X-LINKED IMMUNODEFICIENT MOUSE TO POLYSACCHARIDE VACCINES

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A mutation in the gene encoding Bruton's tyrosine kinase enzyme leads to impaired B cell receptor signaling in Xid mouse. Xid mouse response to T cell independent type 2 (TI-2) antigens such pneumococcal vaccines is severely impaired. The B cell molecule, TACI is critical in the development of antibody response against TI-2 antigens. Here, we found that Xid B cells had significantly reduced levels of TACI as compared to wild type mouse. More importantly, Xid B cells did not secrete immunoglobulins after stimulation with BAFF or APRIL, the two ligands of TACI. Analysis of the signaling cascade induced by BAFF and APRIL showed that while canonical NFkB pathway was blocked, non-canonical NFkB pathway was intact in Xid B cells. These data suggested that reduced TACI expression may be responsible for impaired Ig secretion because TACI mediates signals that activate the canonical NFkB pathway. Since Xid mouse responds poorly to polysaccharide vaccines, it is likely that reduced TACI expression plays an important role in this outcome. The dependence of BAFF and APRIL functions on the expression levels of TACI was further demonstrated in experiments where Xid B cells were pre-stimulated with CpG. Incubation of Xid B cells with CpG increased the expression of TACI and rendered them susceptible to BAFF or APRIL induced Ig secretion. Moreover, BAFF and APRIL induced canonical NFkB pathway activation was restored in CpG pre-stimulated Xid B cells. Finally, immunization of Xid mouse with a prototype TI-2, NP-Ficoll led to the generation of antibodies against NP.

No conflict of interest

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PNEUMOCOCCAL LIPOPROTEINS MODULATE INNATE EFFECTOR FUNCTIONS OF ALVEOLAR MACROPHAGES

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Lipoproteins form a significant proportion of surface exposed proteins of *Streptococcus pneumoniae*. Given their surface localisation, and importance in pneumococcal fitness and virulence we focussed our study on functional characterization of pneumococcal lipoproteins. A pneumococcal strain deficient in lipoprotein diacylglyceryl transferase (lgt), a key enzyme in the lipoprotein biogenesis, was constructed by inframe gene replacement mutagenesis. Deletion of lgt abrogated adhesion to the murine alveolar macrophage cell line MH-S by >50% suggesting the involvement of lipoproteins in pneumococcal adherence. Further the effect of Triton X-114 extracted lipoprotein fraction on innate effector functions of the macrophage was studied. Treatment of MH-S cells with the extracted lipoproteins resulted in 1.5 fold higher reactive oxygen species (ROS) production compared to untreated sample as determined by flow cytometry. A significant increase in nitric oxide (NO) production was observed 24 hours following stimulation of MH-S cells with pneumococcal lipoproteins. The NO production diminished in the presence of inducible nitric oxide synthase (iNOS) inhibitor L-NAME suggesting the involvement of iNOS in pneumococcal lipoprotein mediated NO production. Our data suggests that pneumococcal lipoproteins have important implication in adhesion and modulation of innate effector functions of alveolar macrophage.

No conflict of interest

ISPPD-0301
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NATURALLY ACQUIRED HUMAN IGG PROTECTS AGAINST STREPTOCOCCUS PNEUMONIAE THROUGH RECOGNITION OF PROTEIN RATHER THAN CAPSULAR ANTIGEN

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Background and Aims: Naturally acquired adaptive immunity to *Streptococcus pneumoniae* is partially mediated by antibody, but whether the target antigens are the capsule or sub-capsular protein antigens is not known. We have used human intravenous immunoglobulin preparation (IVIG) to assess the comparative importance of anti-capsule or anti-protein IgG for immunity to *S. pneumoniae*.
Methods: The effects of IVIG were assessed using IgG binding, growth inhibition, agglutination, and phagocytosis assays, and in mouse infection models. The relative contribution of anti-capsule or anti-protein IgG in these assays was assessed using encapsulated and unencapsulated strains, pronase degradation of surface proteins, and IVIG depleted of anti-capsular antibody.
Results: Incubation of live *S. pneumoniae* with IVIG resulted in surface deposition of IgG on the bacteria, increased phagocytosis, growth inhibition, and bacterial agglutination. Results with unencapsulated mutants from 3 different

serotypes showed increased IgG binding, phagocytosis, growth impairment and bacterial agglutination compared to encapsulated strains, indicating these phenotypes are independent of capsule expression. IgG binding to a capsular serotype 4 strain was not affected by depletion of anti-capsular serotype 4 IgG from IVIG, but was reduced on pronase treated *S. pneumoniae*. Passive treatment with IVIG preparations protected mice against septicaemia and pneumonia caused by a serotype 4 strain even after depletion of anti-capsular serotype 4 IgG.
Conclusions: These data suggest that naturally acquired adaptive immunity to *S. pneumoniae* is largely dependent on IgG that recognises protein antigens rather than the capsule, which has important implications for preventative strategies versus *S. pneumoniae* infections.

No conflict of interest

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SEROTYPE DISTRIBUTION AND VIRULENCE PROPERTIES OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM COMMUNITY AND HOSPITAL: A RETROSPECTIVE STUDY IN NORTH INDIA

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Background and Aim: Nasopharyngeal colonization plays an important role in the development and transmission of pneumococcal diseases. The knowledge of serotypes actually responsible for disease is essential for effective disease management and vaccine implementation. Hence, we have assessed the pneumococcus serotypes accountable for disease and their virulence pattern in comparison to carrier strains.

Methods: Clinical strains (n = 20) were obtained from (OPD, PGIMER) sputum, nasopharyngeal and blood samples of patient's suffering from respiratory diseases. Carrier strains were already collected from community based surveillance. The isolates were serotyped by multiplex PCR and further confirmed by sequencing. The virulence was determined in terms of adherence pattern and cytopathic effect in cell lines.

Results: Clinical strains from nasopharyngeal and sputum samples possessed mostly 19F serotype whereas isolates from blood samples belonged to serotypes 1, 7C/B, 22F/A, 10A and 23F. In our previous study, we have shown the presence of serotypes 7C/B (2.9%), 22F/A (1.0%), 10A (4.8%) and 23F (3.8%) in asymptomatic healthy children along with 6A/B/C, 11A/D, 15B/C, 10F/C, 34, 10A being the most prevalent. The adherence capability of clinical strains was almost 2 fold higher as compare to carrier strain with blood isolates being most virulent. The carrier strain or sputum isolates did not show any significant cytotoxic effects on human cell lines.

Conclusions: We found that disease causing serotypes parallelly exist in asymptomatically healthy children and serotype variation among strains reflected in their virulence capabilities. We are further characterizing the pneumococcal surface proteins which could explain the differential adherence properties of carrier and clinical strains.

No conflict of interest

ISPPD-0457
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THE EFFECT OF MULTIPLE FREEZE/THAW CYCLES ON THE DETECTION OF ANTI-STREPTOCOCCUS PNEUMONIAE IGG AND THE FUNCTIONAL ACTIVITY OF SERUM

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Background and Aims: With the increasing number of vaccines being delivered in infancy, clinical trials of new vaccines designed for the infant immunisation programme require the immune response to both the investigational and concomitant vaccines to be measured. With serum volumes limited in infant trials, samples may require recurrent freeze thaw cycles during the testing phase as assays are prioritised. Historical concerns about the deterioration in the quality of sub-optimally stored serum and samples undergoing repeated freeze thaw cycles has led to limits being imposed. We sought to test the impact of repeated freezing and thawing of serum on pneumococcal IgG quantitation and opsonophagocytic killing to establish whether such concerns are justified.
Methods: A set of 12 pneumococcal Quality Control samples stored at -80°C were thawed and frozen 20 times. Sera were assayed after 0, 1, 2, 4, 8, 12, 16 and 20 freeze/thaw cycles for serotype specific IgG (13 serotypes by ELISA) and functional killing ability (4 serotypes by an Opsonophagocytic Assay).
Results: Analysis of the IgG values for the 13 serotypes revealed remarkable stability. The mean ratio of IgG after 20 FT cycles compared to the starting value ranged from 0.97 (18C) -1.1 (6A). For the OPA, results after 20 cycles were within a 3-fold titer compared to the original for all 11 sera and 4 serotypes tested. A 3 fold variation is within the acceptance criteria of the assay for a given serum.
Conclusion: Immune responses specific for the pneumococcus appear robust and able to withstand repeated freeze thaw cycles.

No conflict of interest

ISPPD-0548
Man versus Microbe---who gets pneumococcal disease and why?

PERSISTENCE OF ANTIBODY LEVELS FOLLOWING ROUTINE INFANT IMMUNIZATION WITH THE 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE

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Background and Aims: Determining the longevity of serotype-specific antibodies induced by pneumococcal conjugate vaccines (PCVs) is important for understanding the potential long-term effectiveness of routine PCV use.
Methods: An observational, prospective, longitudinal study of nasopharyngeal carriage consisting of monthly visits for 6 months was conducted among American Indian households in the Southwest United States from 2006-2008 to evaluate the impact of long-term PCV7 routine use. Unimmunized children were age-matched to those immunized with PCV7 at least four years prior (ratio 1:4). Blood collected at the final study visit was analyzed by ELISA for PCV7 serotype IgG. Geometric mean concentrations (GMCs) and the odds of having ≥ 0.35 µg/mL of serotype specific antibody were compared according to immunization status using a matched regression approach.
Results: Eight unimmunized children (mean age: 7.9 years) were age-matched to 28 immunized children at the time of serum collection (mean age: 7.9 years). Serotype specific GMCs were comparable for six of the seven serotypes between immunized and unimmunized subjects; the serotype 14 GMC was significantly lower for unimmunized (immunized: 0.7 vs. unimmunized: 0.2; $p = 0.02$). There was no difference in the odds of the immunized and unimmunized children reaching or exceeding 0.35 µg/mL, the vaccine licensure correlate threshold.
Conclusions: Four years following routine infant immunization, serotype-specific IgG levels were similar to those of age-matched unimmunized children. Natural exposure to pneumococcus may be critical in inducing or maintaining persistent antibody and thus the elimination of circulating pneumococci by PCV may have profound effects on long-term immunity.

No conflict of interest

ISPPD-0555
Man versus Microbe---who gets pneumococcal disease and why?

PNEUMOCOCCAL ANTIBODY LEVELS OF CHILDREN IN JAPAN BEFORE THE INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINE

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Streptococcus pneumoniae remains a leading cause of serious illness, including bacteremia, meningitis, and pneumonia among children. In Japan, 7-valent pneumococcal conjugate vaccine (PCV7) was first approved in 2009, and was introduced to routine immunization program for infant in 2013. Thus, significant decrease of invasive pneumococcal diseases (IPD) has been expected, however, the serosurveillance data before the introduction of PCV7 in children is lacking, and it is difficult to evaluate the impact of PCV in Japan. In this study, serosurveillance was conducted by measuring serotype-specific antibody and opsonophagocytic index (OI) of sera collected from children before the introduction of PCV7 in Japan. Serotype-specific antibodies in each age-group were enough high as the infection prevention threshold (0.2 µg/ml) against most of serotypes, however, OI in each age-group were separated into 2 groups (high/low OI groups). High groups contained OI more than 8, which is comparable to the infection prevention threshold of serotype-specific antibody concentration, and low groups contained relatively low OI (OI<8). This trend was rather evident in samples of 1- and 3-year-old children. Amount of serotype-specific antibodies did not correlate with OI, and older children naturally possess higher OI than younger children without PCV vaccination.
This serosurveillance suggested that pneumococcal vaccination program, which has been launched in Japan, is reasonable and strongly required in children, mainly for low OI group, to induce protective immunity against pneumococcal infections. Now, serosurveillance after the introduction of PCV in Japan is evaluating its impact in terms of immune response for the protection against IPD.

No conflict of interest

ISPPD-0235
Man versus Microbe---who gets pneumococcal disease and why?

SEROLOGIC RESPONSE TO PNEUMOCOCCAL VACCINATION IN CHILDREN EXPERIENCING REPETITIVE INVASIVE PNEUMOCOCCAL DISEASE

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Background and Aims: Some children are prone to recurrent invasive pneumococcal disease (rIPD) and of these, some respond insufficiently to standard pneumococcal vaccination. Little is known about how to handle these children and if they benefit from additional vaccination. Here, we present preliminary results from a nationwide study of paediatric rIPD including data on serotype-specific vaccination response to pneumococcal polysaccharide vaccination (PPV23) and pneumococcal conjugate vaccination (PCV7).
Methods: A retrospective, population-based study was conducted using The National *Streptococcus pneumoniae* Registry, which contains laboratory-confirmed data from all cases of IPD in Denmark. From January 1980–June 2013 all children aged 0–15 years with rIPD were identified. Clinical data and data on serotype-specific pneumococcal antibody response were collected. Over the years quantification of pneumococcal antibodies varied from being presented in arbitrary units (ELISA), in µg/ml (WHO ELISA) and lately in µg/ml based on Luminex technology.
Results: 2192 children were diagnosed with IPD and 75 episodes of rIPD were documented in 59 children. An underlying disease was documented in 39 (67%) children. 22 children were vaccinated; 11 solely with PPV23, 6 with PPV23+PCV7 and 5 with PCV7. In total, 13 responded to vaccination and 9 were non-responders. Among PPV23 non-responders, 3 responded to PCV7 vaccination.
Conclusions: In our study 41% of the children with rIPD responded insufficiently to pneumococcal vaccination. PPV23 non-responders benefitted from PCV vaccination.

No conflict of interest

ISPPD-0065
Man versus Microbe---who gets pneumococcal disease and why?

IMPACT OF IGM ON FUNCTIONAL IMMUNE RESPONSES TO SEROGROUP 6 AND 19 AFTER IMMUNIZATION WITH PCV7 IN CHILDREN

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Background and Aims: To investigate the impact of IgM on functional immune responses to vaccine-related serotypes 6A, 6C and 19A as well as vaccine serotype 6B and 19F in children after immunization with 7-valent pneumococcal conjugate vaccine (PCV7).
Methods: Eighteen immune sera were obtained from children aged 12-23 months after complete immunization (3+1) with PCV7. The serum opsonic indices (OI) to serotypes 6B and 19F and serotypes 6A, 6C and 19A were studied by opsonophagocytic killing assay (OPA). The role of IgM antibodies on opsonic indices of immunized sera were determined by measuring OI after depletion of IgM antibody of immune sera. The specificity of antibodies (IgG or IgM) to each serotypes was studied with competitive OPA to 6B, 6A, 6C, 19F and 19A pneumococci with 6B polysaccharide or 19F polysaccharide, respectively.
Results: Compared to control sera, OI of IgM-depleted immune sera to 6B and 19F were decreased in 94% or 71% of subjects, respectively. OI to 6A, 6C, and 19A were decreased in 92%, 100% or 91% of sera after IgM depletion, too. In competitive OPA, free 6B or 19F polysaccharide completely inhibited the immune protection to serotypes 6A, 6C, and 19A as well as serotypes 6B and 19F in most samples.
Conclusion: The booster immunization of PCV7 certainly induced cross-protective antibodies to vaccine-related serotypes 6A, 6C, and 19A with both IgG and IgM isotypes. The IgM antibodies in children after complete immunization of PCV was highly contributed to opsonophagocytic activity to vaccine-related serotypes as well as vaccine types.

No conflict of interest

ISPPD-0012
Man versus Microbe---who gets pneumococcal disease and why?

ADENYLATE KINASE REQUIRED FOR PNEUMOCOCCAL GROWTH AND CAPSULAR SYNTHESIS

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Streptococcus pneumoniae (pneumococcus) infection causes high mortality and morbidity worldwide. Capsular polysaccharides (CPS) are major virulence factors by protecting pneumococci from host. Synthesis of CPS requires energy supplied by ATP hydrolysis, and adenylate kinases (AdKs) constitute a major family of enzymes to regulate cellular ATP level. However, it remains poorly understood whether AdK acts as a virulence factor in pneumococcal diseases. Here we show that AdK from *S. pneumoniae* (SpAdK) is essential for growth, CPS synthesis and bacterial survival *in vivo*. Expression of the wild-type *adk* gene in fucose-inducible strains rescued growth defect. Cellular ATP and CPS levels were increased in proportion to the expression level of *adk* gene. Taken together, our results support that SpAdK is required for pneumococcal growth and CPS synthesis. We propose that SpAdK is a novel pneumococcal virulence factor.

No conflict of interest

ISPPD-0340
Man versus Microbe---who gets pneumococcal disease and why?

IMMUNE RESPONSE TO SEVEN-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN CHILDREN WITH INVASIVE PNEUMOCOCCAL DISEASE

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Background and Aims: Immune responses to pneumococcal conjugate vaccine (PCV) have not been fully investigated in children with invasive pneumococcal diseases (IPD). The aim of this study is to investigate the antibody responses to seven-valent PCV (PCV7) in children with IPD
Methods: Of fifty-six children diagnosed with IPD between October 2009 and April 2013 in Japan, 17 children were examined for the geometric mean concentration of serotype-specific IgG and the geometric mean titers of opsonization indices (OIs) using paired sera obtained at the onset of IPD and after the last PCV7 dose.
Results: The GMCs of serotype-specific IgG for all PCV7 serotypes other than serotype 6B were significantly increased after the last PCV7 dose compared with those at the time of IPD onset ($p < 0.01$), as were the GMTs of OIs for all PCV7 serotypes. In 14 children with IPD caused by PCV7 serotypes for whom both IgG and OI results were available, the OIs for the infecting serotype were undetectable, although the IgG levels varied between from <0.2 to >5.0 μg/ml at the time of IPD onset. After the last PCV7 dose, the OIs for the infecting serotype were undetectable (OI < 8) for six (43%) of 14 children. Unresponsiveness was found in children with IPD caused by serotype 6B (n = 5) and serotype 23F ($n = 1$).
Conclusion: Immune unresponsiveness after PCV7 was specific for the infecting serotype. Careful monitoring of the OI for the infecting serotype after vaccination with PCV is required in children who experienced IPD.

No conflict of interest

ISPPD-0094
Man versus Microbe---who gets pneumococcal disease and why?

PROTECTIVE EFFECTS OF ORAL ADMINISTRATION OF LACTOBACILLUS DELBRUECKII SSP: BULGARICUS OLL1073R-1 WITH EXOPOLYSACCHARIDES IN PNEUMOCOCCAL PNEUMONIA IN MICE

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Background and Aims: Intestinal flora is a major modulator of systemic immunity and oral administration of probiotics has been known to improve inflammatory responses against infectious diseases. *Lactobacillus delbrueckii ssp. bulgaricus* OLL1073R-1 (1073R-1) was reported to enhance the effects of the innate immune system and the exopolysaccharides (EPS) produced by 1073R-1 are thought to play an active role in enhancing immunostimulatory effects. We show the protective effect of pre-administration of 1073R-1 and EPS against pneumococcal pneumonia in mice.
Methods: 1073R-1 and EPS were orally administered to C57BL/6 mice for 21 days prior to intranasal infection with *Streptococcus pneumoniae* (strain D39).
Results: The mice treated with 1073R-1 and EPS were prone to prolonging the duration of survival and inhibiting

the body weight loss, compared to control mice however there were no significant differences. Bacterial counts in the lung were significantly reduced in 1073R-1 and EPS treated mice compared to control mice (4.123±0.2955 and 5.074±0.2878 (log colony forming units (CFU)/ml) respectively: P=0.0416). The levels of interleukin-6 (IL-6) in the lung were significantly reduced in 1073R-1 and EPS treated mice compared to control mice (13.47±5.559 vs 140.3±62.27 pg/ml, respectively: p = 0.0475). In addition the significant reduction of tissue injury and inflammatory cell accumulation was observed in 1073R-1 and EPS treated mice compared to control mice in histopathological analysis of the lungs
Conclusions: Probiotics by 1073R-1 and EPS could be one of the useful tools to protect host from the pneumococcal infection.

Conflict of interest

ISPPD-0442
Man versus Microbe---who gets pneumococcal disease and why?

HIGH PREVALENCE OF IMMUNOCOMPROMISING CONDITIONS AMONG YOUNG ADULTS PRESENTING WITH INVASIVE PNEUMOCOCCAL DISEASE IN A SCOTTISH INNER CITY AREA

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Introduction: Invasive pneumococcal disease (IPD) is relatively uncommon in young, otherwise healthy adults. HIV infection and other medical conditions conferring immunocompromise are known to be associated with an increased risk of IPD. National guidelines suggest testing patients with bacterial pneumonia and meningitis for HIV but uptake of testing for this indication in our area is not known.
Methods: Patients aged between 18 and 45 years presenting between 2008 and 2012 to North Glasgow Hospitals with a blood or cerebrospinal fluid culture positive for *Streptococcus pneumoniae* were included. Laboratory and clinical records were reviewed to establish if there was an existing history of immunocompromise and whether further testing was undertaken.
Results: 63 patients met the inclusion criteria; 34 (54%) male. Most cultures were taken from emergency departments and admission units (44/63, 70%). 10 patients (16%) had a pre-existing diagnosis of a condition likely to lead to immunocompromise (5 (8%) HIV, 3 (5%) solid organ malignancy receiving chemotherapy, 2 (3%) haematological malignancy. Of the remaining 53 patients, testing was undertaken for HIV (10 patients (19%)), immunoglobulins (9 patients (17%)) and functional antibodies (6 patients (11%)). As a result of this testing, 5 patients were found to have significant abnormalities: HIV, multiple myeloma secondary hypogammaglobulinaemia and low anti-pneumococcal antibodies (2 patients).
Conclusion: Young adults presenting with IPD represent a group who have a high prevalence of immunocompromise. The most common underlying condition was HIV. However, most patients with IPD in our area are not tested for HIV or other IPD risk conditions.

No conflict of interest

ISPPD-0426
Man versus Microbe---who gets pneumococcal disease and why?

MEMORY T CELL PROLIFERATIVE RESPONSES TO PNEUMOCOCCI ARE REDUCED IN OLDER ADULTS

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Introduction: Immunity mediated by $CD4^+$ cells, in particular the Th17 subset, has been implicated in the acquired immune response to pneumococcal disease. Age-related changes in this immunity are not known.
Methods: Peripheral blood mononuclear cells were isolated from healthy volunteers and patients with recent invasive pneumococcal disease. $CD14^+$ monocytes and $CD4^+CD45RO^+$ memory lymphocytes were isolated by magnetic bead selection. Memory $CD4^+$ lymphocytes were co-cultured with monocytes before being infected with pneumococci (TIGR4) or heat inactivated Candida albicans (HKCA). Further microbial growth was inhibited with antibiotics. After 7 days, cultures were stimulated with PMA and ionomycin in the presence of brefeldin A and stained for intracellular cytokines.
Results: T cell proliferation in response to pneumococcal stimulation was monocyte dependent. Proliferating lymphocytes expressed IL-17A, IL-17F, IL-22 and interferon-g but not IL-4. When older adults (>65 years) were compared with younger adults (<35 years), total proliferation and proliferation of interferon-g and IL-17A expressing cells were significantly reduced when stimulated with TIGR4 but not when stimulated with HKCA. Resultant levels of secreted IL17A and interferon-g were also lower in older adults compared with younger adults. Patients with a history of recent invasive pneumococcal disease also had reduced proliferation of lymphocytes in response to stimulation with TIGR4 compared to matched controls.
Conclusion: Among healthy volunteers, older adults and patients with recent invasive pneumococcal disease have reduced proliferation of interferon-g and IL-17A producing lymphocytes. This reduced Th17 immunity may account for the increased risk of pneumococcal disease in the elderly and is a target for vaccination.

No conflict of interest

ISPPD-0240
Man versus Microbe---who gets pneumococcal disease and why?

EFFECT OF AGEING ON HUMORAL AND CELLULAR IMMUNE RESPOSNES TO PNEUMOCOCCAL CONJUGATE AND POLYSACCHARIDE VACCINATION IN MICE

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Background and Aims: Ageing is associated with diminution of immune responses and increased susceptibility to invasive pneumococcal disease and pneumonia. We studied cellular and humoral immune responses to pneumococcal conjugate (PCV) and polysaccharide (PPS) vaccination in young and aged mice of two different genetic backgrounds.
Methods: Young (3-4 mo) and aged (15-16 mo) CD-1 and Balb/c mice were immunized with saline or with one dose of (PPS; Pneumovax or PCV; Prevenar7). Concentrations of IgM and IgG antibodies to pneumococcal polysaccharide and protein antigens were measured by Luminex at 7-days post-vaccination. Induction of pro-inflammatory and non-inflammatory cytokines was measured by Luminex after in vitro stimulation of peripheral blood mononuclear cells (PBMC) with different antigens.
Results: Age-, mouse strain-, and serotype-specific differences in antibody responses were detected at post-vaccination. Low concentrations of IgM antibodies to several pneumococcal protein antigens were detected in all treatment groups, including sham-injected animals, with no significant changes after PCV or PPS vaccination. PBMC stimulation with PCV and heat-killed pneumococcus, but not *Haemophilus influenzae*, induced IL-17A responses especially in the aged CD-1 mice.
Conclusion: Aging affected both humoral and cellular immune responses to pneumococcal vaccination in mice but the genetic background of the mice played also a significant role.

No conflict of interest

ISPPD-0311
Man versus Microbe---who gets pneumococcal disease and why?

THE BURDEN OF INVASIVE PNEUMOCOCCAL DISEASE [IPD] IN MASSACHUSETTS' CHILDREN WITH UNDERLYING CONDITIONS

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Background: Despite the success of pneumococcal conjugate vaccine (PCV), children with underlying conditions remain at increased risk of IPD.
Methods: Cases of IPD in children <18 years of age were detected through an enhanced surveillance system in MA. *Streptococcus pneumoniae* (SP) isolates, when available, are submitted to MDPH and parents/physicians are interviewed for demographic and clinical data. Isolates are confirmed as SP, serotyped with Quelling reaction. Underlying conditions were classified using 2012 Red Book risk categories.
Results: Between April 1, 2002 and September 30, 2013, 1025 cases of IPD have been identified in MA children <18 years of age; 196 (19.17%) had comorbidities; conditions associated with immunosuppression were most frequent (64/196;32.6%). IPD cases in children with comorbidities were older [67.3 mo.(95%CI 58.9-75.6) vs. 42.7 mo. (95%CI 39.4-46.0)($p < 0.001$)]; had more frequent hospitalization [odds ratio (OR) 2.9(95%CI 1.9-4.3)]; and demonstrated increased case fatality [OR 2.5(95%CI 1.0-5.9)]. Clinical presentations were not different among cases with comorbidities; however bacteremic pneumonia was observed more often in children with asthma [OR 2.7 95% CI 1.2-6.1)]. 803 isolates (78.3%) were available for serotyping; most common serotypes were 19A (254/803;31.6%), 7F (116/803;14.5%), 22F(42/803;5.2%) and 33F(40/803;4.9%). Nonvaccine serotypes (NVST) contributed to higher proportion of IPD in children > 5 yrs. with comorbidities (36/71;50.7% of cases >5 years vs. 36/90;40.0% <5 years of age).
Conclusion: IPD in children with comorbidities was associated with higher hospitalization and case fatality rates. NVST was more prevalent as the cause of IPD in children >5 years with comorbidity. Children with asthma presented with bacteremic pneumonia more frequently compared to children without comorbidity.

No conflict of interest