O451
1-hour Oral Session
New insights into host-pathogen interactions

Phage-derived gene is involved in platelet activation and associated with increased mortality in patients with invasive pneumococcal disease

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Background: *Streptococcus pneumoniae* is a Gram-positive bacterium causing severe invasive disease. Despite the identification of a whole range of different virulence factors playing a role at different stages of infection, the mechanisms underlying pathogenesis are not fully understood. To improve our understanding about the severity of disease in invasive pneumococcal disease (IPD) we investigated the association between pneumococcal genotype, based on whole genome sequences of 349 pneumococcal blood isolates, and mortality, based on clinical data from 349 bacteremic patients.

Material/methods: Consecutive patients hospitalized with a bacteremic pneumococcal infection at two Dutch hospitals between 2001 and 2011 were included in the study. Corresponding blood culture isolates of *S. pneumoniae* were collected and serotyped. Putative protein coding sequences were investigated using an “all-versus-all” protein BLAST (blastP), with a 10e-15 *e*-value cut-off and a BLOSUM90 substitution matrix. The results were subsequently clustered into OGs using TribeMCL, resulting into a total of 3021 orthologous genes (OGs). Associations between the presence or absence of pneumococcal OGs and 30-day mortality were investigated. We proceeded to induce the expression of the phage-encoded gene which was found to be associated with 30-day mortality, and subsequently performed *in vitro* assays to investigate its consequence on platelet activation by *S. pneumoniae*.

Results: We observed a strong statistical correlation between 30-day mortality and the presence of the phage-encoded gene *pblB*; 81% in the pneumococcal isolates derived from the deceased patients versus 45% in the isolates from patients that survived IPD. The presence of *pblB* was an independent

determinant of 30-day mortality, irrespective of other clinical factors. Higher *pblB* gene expression was found to be induced by the fluoroquinolones ciprofloxacin and levofloxacin. Subsequently, we demonstrated that high expression of *pblB* induced with levofloxacin activate platelets more significantly as opposed to the non-induced pneumococci.

Conclusions: Utilizing the power of genome sequencing and GWAS, the phage-encoded gene *pblB* was found to be associated with 30-day mortality in patients with IPD. Induction of the *pblB* phage in pneumococci resulted in higher platelet activation *in vitro*, and this may help explain the increased mortality in IPD patients. We believe that this integrated approach will assist greatly in elucidating the mechanisms of various bacterial pathogenesis leading to the development of novel diagnostics and new therapeutic approaches.