from BR patients (n = 115), categorised by bronchiectasis severity index (BSI) scores and sera samples from HV controls (n = 26).

**Results** Endobronchial biopsies from BR airways had a significantly (p < 0.05) higher number of blood vessels per mm of basement membrane than HV samples (18 and 9 blood vessels/mm basement membrane respectively). Stimulation of HV neutrophils with a variety of molecules (PMA, fMLP, LPS, TNF-α etc.) resulted in a significant increase in VEGF secretion compared to unstimulated (p < 0.05). Although elevated VEGF was found in some patient samples there was no significant correlation between sera/spuva VEGF and individual patient BSI scores.

**Conclusion** The increased presence of vascular tissue seen in BR could indicate a pro-angiogenic airway environment in BR. The in vitro data collected also show that a variety of stimulants can initiate secretion of VEGF by neutrophils. However, our data does not suggest that VEGF levels in sera or spuva can be used to predict disease severity.

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**PNEUMOOLYSIN PROMOTES NEUTROPHIL: PLATELET AGGREGATION IN VITRO**

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10.1136/thoraxjnl-2016-209333.53

**Introduction and objectives** The pneumococcal cholesterol-binding, pore-forming toxin, pneumolysin (Ply), appears to be a key mediator not only of the acute lung injury, but also myocardial damage, associated with severe pneumococcal disease. Although direct Ply-mediated cardiopulmonary toxicity has been implicated, the neutrophil- and platelet-targeted pro-inflammatory activities of the toxin are also believed to contribute to the pathogenesis of these adverse events, albeit by poorly characterised mechanisms. To test the hypothesis that Ply promotes neutrophil: platelet networking, we have investigated the effects of the toxin on the induction of heterotypic aggregation of these cells *in vitro*.

**Methods** Neutrophil: platelet-enricheduffy coat suspensions were prepared from the heparinised blood of healthy, adult humans by sedimentation (at 37°C) and diluted 1:50 in Hank’s balanced salt solution. Following 5 min of preincubation, recombinant PAF (10–80 ng/ml), or the pneumolysoid, delta 6Ply (attenuated with respect to pore-forming activity, negative control), or adenosine 5'-diphosphate (ADP 100 μM, positive control) were added to the cell suspensions. After a further 5 min period of incubation at 37°C, samples were stained with 5 μl of each of the following murine, anti-human, fluorochrome-labelled monoclonal antibodies: CD16-APC (neutrophils), CD42a-PE (platelets), and CD45-Krome Orange, and incubated for 15 min at room temperature in the dark. This was followed by analysis of samples at a slow rate using a Gallios flow cytometer. The relative numbers of platelets interacting with a single neutrophil were determined using the relative mean fluorescence intensities of CD16+/CD42a+/CD45+ neutrophils.

**Results** These are shown in the accompanying table. Addition of PAF to the mixed cell suspension resulted in statistically significant dose-related formation of neutrophil:platelet aggregates which was maximal at 80 ng/ml and greater in magnitude to that observed with ADP, while delta6Ply was ineffective.

**Conclusion** PAF, at pathologically-relevant concentrations, promotes neutrophil: platelet aggregation *in vitro*, an activity which is dependent on the pore-forming properties of the toxin. Given the increasing recognition of the role played by platelets in driving neutrophilic inflammation, this activity of Ply may exacerbate pulmonary and myocardial injury in severe pneumococcal disease.