The inflammatory response to bacteria requires the interaction of pattern recognition receptors with bacterial surface constituents, and humans deficient in components of inflammatory signalling pathways such as IRAK4 are prone to invasive pneumococcal disease. Pneumolysin is a well-recognised virulence factor for Streptococcus pneumoniae that has multiple effects on the host immune response that are primarily thought to be pro-inflammatory; including causing IL1β release due to pore formation, and epithelial cell layer breakdown. We hypothesised that pneumolysin deficient TIGR4 (a serotype 4 strain) would induce less inflammatory cytokines than wildtype from human monocyte derived macrophages. While both pore forming and non-cytolytic purified pneumolysin induced dose dependent inflammatory cytokine release, the pneumolysin deficient bacteria induced greater TNF and IL6 than wildtype, by qPCR and ELISA measurement of protein. This was reduced by inhibition of phagocytosis with cytochalasin D. Given the pore forming effects of pneumolysin we assessed whether differential cell death contributed to the differences in inflammatory response. While wildtype bacteria caused more cell death at 24 h, inhibition of caspases had no effect on the cytokine response suggesting that apoptosis pathways don't directly influence the early inflammatory response. Transcriptome analysis confirmed increased pro-inflammatory and interferon gene signalling with the mutant strain, with reduction of the inflammatory and interferon signature with inhibition of phagocytosis. Wildtype bacteria induced less NFκB translocation, but more IRF3 translocation than Δ*ply*. An *in vivo* intranasal mouse infection showed wildtype was more virulent, with more bacteria recovered from bronchoalveolar lavage fluid at 4 h. However, this was associated with reduced TNF compared to Δ*ply*. Neutralising TNF intranasally abrogated the difference in bacteria recovered between wildtype and Δ*ply*. Thus, the early inflammation dampening effects of pneumolysin released within the phagolysosome may be an important contribution to its virulence by allowing bacterial replication at mucosal surfaces. This may be due to IRF3 mediated inhibition of inflammatory cytokine transcription. Better understanding of the biology of pneumolysin may aid in adjuvant treatment of *S. pneumoniae*.

# New developments in cough

## S87 ARE ORAL STEROIDS EFFECTIVE IN TREATING THE SYMPTOMS OF ACUTE LOWER RESPIRATORY TRACT INFECTION IN NON-ASTHMATIC ADULTS? THE ORAL STEROIDS FOR ACUTE COUGH (OSAC) PLACEBO-CONTROLLED RANDOMISED TRIAL

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10.1136/thoraxjnl-2015-207770.93

**Background** The majority of UK adults experience at least one lower respiratory tract infection (LRTI, or acute bronchitis) a year. Despite an absence of evidence in this patient group, some GPs prescribe inhaled or oral corticosteroids. OSAC sought to demonstrate 'proof of concept' symptomatic effectiveness of a moderate dose of oral corticosteroid for adults without asthma or COPD with acute LRTI.

**Methods** OSAC was a double blind, placebo controlled RCT set in GP practices in England, powered to investigate if oral prednisolone reduces the duration of moderately bad or worse cough and/or the severity of its associated symptoms, when compared to placebo, by at least 20%. Adults (≥18 years) with acute (≤28 days) cough, for whom same-day antibiotics were not clinically indicated, and without asthma or COPD, received 40 mg oral prednisolone or matched placebo for 5 days. Symptom diaries, completed for up to 28 days, measured two primary outcomes: the duration of moderately bad or worse cough; and the average severity of all symptoms on days 2 to 4 on a scale of 0–6. We sought to demonstrate a minimum clinically important reduction of 20% in each outcome.

**Results** 398 participants were randomised to either prednisolone or placebo tablets (198 and 200 respectively) from 54 UK primary care sites. Attrition was lower than expected, giving over 85% power for the two primary outcomes. Data were analysed on an intention-to-treat basis. The median duration of moderately bad or worse cough was 5 days in both groups (IQRs 2–8 and 3–8 for prednisolone and placebo respectively). Adjusting for trial centre and baseline characteristics, this gave a hazard ratio of 1.11 (95% CI 0.89 to 1.39, p = 0.35). Symptom severity was lower in the prednisolone group (mean 1.99 vs 2.16), adjusted difference -0.090 (-0.212 to 0.003, p = 0.152).

**Conclusions** We found no evidence that a moderately high dose of oral corticosteroid reduced either duration of moderately bad (or worse) cough, or symptom severity at days 2 to 4 in adults without asthma or COPD with LRTI not requiring immediate antibiotic treatment. Lower dose oral or high dose inhaled corticosteroids are also unlikely to be beneficial.

## S88 THE VIRAL MIMIC POLYINOSINIC: POLYCYTIDYLIC ACID (POLY I:C) INDUCES TRPA1 CHANNEL HYPER-RESPONSIVENESS IN AN ADULT HUMAN STEM CELL-DERIVED SENSORY NEURONAL MODEL

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10.1136/thoraxjnl-2015-207770.94

**Background** Changes in airway neuronal activity are likely to underpin the heightened irritant responses such as excessive cough and wheeze which accompany respiratory virus induced exacerbations of airways disease. The mechanisms responsible are unknown but we hypothesised that neurons express pathogen recognition receptors such as toll-like receptors (TLR) through which viruses may alter neural function. Investigating this is hampered by the lack of suitable human tissues with both nerve endings and cell bodies present. We have refined an adult human neural crest stem cell-derived sensory neuronal model to overcome this obstacle.

**Methods** Human dental pulp stem cells (hDPSCs) were differentiated towards a neuronal phenotype, termed peripheral neuronal equivalents (PNEs). Using molecular and immunofluorescent techniques, together with whole cell patch clamp electrophysiology, we investigated the expression and function of TLRs and the transient receptor potential (TRP) channels TRPV1 and TRPA1 on PNEs. We then assessed the effects of exposure to a viral mimic, the synthetic TLR3 agonist (polyI:C), on cytokine

release and changes in the PNE responsiveness to the TRPA1 channel agonist cinnamaldehyde (100 μM).

**Results** hDPSCs undergo a fibroblastic to neuronal phenotypic switch to PNEs which express the sensory neuronal proteins SP and CGRP. Using qPCR we confirm that PNEs express TLR3, TLR4 and TLR7 mRNA and functional expression of TRPA1 and TRPV1 channels. PNEs pre-treated with PolyI:C (2 μg/ml) for 20 mins generated significantly larger inward (-10.8773 pA/pF; $p < 0.01$) and outward (10.0507 pA/pF; $p < 0.01$) currents in response to cinnamaldehyde (100 μM) compared to untreated PNEs (-2.347 pA/pF and 2.872 pA/pF respectively). The electrophysiological events elicited by PolyI:C occurred rapidly, were not sustained and appeared independent of alteration in TRP channel gene expression. PNEs incubated with PolyI:C for 24 h released significantly greater IL6 (246.504 pg/ml; $p < 0.01$) and IL8 (2140.83 pg/ml; $p < 0.001$) levels compared to untreated PNEs.

**Conclusion** Using a novel human *in vitro* sensory neuronal model we observed that Poly I:C evoked sensory neuronal hyper-responsiveness with an accompanying pro-inflammatory response. Respiratory viruses may induce similar effects on sensory neurons during exacerbations of airways disease.

## S89 HYPERSENSITIVITY TO ADENOSINE TRIPHOSPHATE IN CHRONIC COUGH PATIENTS

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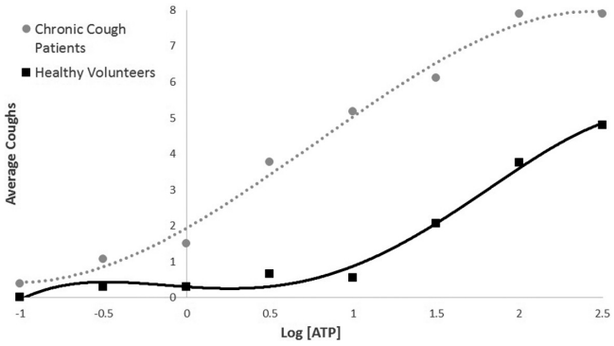
10.1136/thoraxjnl-2015-207770.95

**Introduction** Recent studies have suggested a role for adenosine triphosphate (ATP) activated P2×3 receptors in the pathophysiology of chronic cough. ATP has previously been used as an inhalational challenge substance in asthmatics and COPD patients, with the main focus being on bronchospasm. We have considered whether chronic cough patients are hypersensitive to inhaled ATP compared to healthy volunteers.

**Method** The recognised ERS standardised cough challenge using the Ko-Ko digidoser was performed with ATP and AMP as substrates. 20 Healthy volunteers and 20 chronic cough patients were randomised to the order of challenges. C5 (the concentration of substrate causing at least 5 coughs) was compared for ATP and AMP. Average cough response to ATP was compared between the 2 groups.

**Results** 6 male and 14 female volunteers in each group were randomised to receive cough challenges. Hull Airways Reflux Questionnaire score range was 0–8 in healthy volunteers and 21–52 in chronic cough patients. 1 healthy volunteer had a mild hypersensitivity reaction to ATP with urticaria. 1 patient withdrew after their first challenge due to worsening cough. No other side effects were reported. 2/19 healthy volunteers coughed with AMP, neither achieved C5. 8/20 chronic cough patients coughed with AMP, 2 achieved C5. 18/20 healthy volunteers coughed with ATP with 15 achieving C5. 19/19 chronic cough patients coughed with ATP, 18 achieved C5. The chronic cough patients had a greater cough response at lower concentrations of ATP as demonstrated in Figure 1.




**Abstract S89 Figure 1** Average cough response to ATP in healthy volunteers and chronic cough patients

**Discussion** Previous human ATP challenges have documented cough as a symptom but none have objectively measured the cough response in chronic cough patients. We present here the first results demonstrating that chronic cough patients have increased sensitivity to ATP compared to healthy volunteers. This supports a role for ATP driven receptors in the cough reflex arc and supports further research in this area as a target for treatments in chronic cough.

## S90 'CHRONIC COUGH, CAUSE UNKNOWN': A QUALITATIVE STUDY OF PATIENT PERSPECTIVES OF IDIOPATHIC COUGH

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10.1136/thoraxjnl-2015-207770.96

**Introduction and objectives** Idiopathic chronic cough patients have symptoms that persist despite trials of empirical treatment with no underlying cause found. Higher-order brain processes are involved in modulating the cough reflex, but very little is known about the psychological processes underlying idiopathic cough. As the first step in the development of a complex intervention, we sought to elicit an in-depth understanding of patient experience of this condition.

**Methods** Fourteen patients (12 females, mean age = 59 years) participated in qualitative interviews theoretically based upon the comprehensive cognitive-behavioural model. Interviews were thematically analysed and cross-validated using the guidelines outlined by Braun and Clark (2006).

**Results** Eight key themes emerged illustrating the complex, all-encompassing nature of idiopathic cough. 'Individual vulnerability' described precipitating factors possibly linked with cough onset. 'More than just a cough' highlighted the co-occurrence of severe physical and emotional experiences. 'Cough in the social sphere' highlighted the effort of dealing with others' reactions and concerns about the contagious image. 'Cough and Identity' described how the cough often defines the person. The occurrence of 'Vicious circles' became apparent, contributing to cough maintenance. 'The battle for control' highlighted the unpredictable nature of the cough, its subsequent impact and the management strategies employed to counter this. Framing the 'Cough in