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Thorax

ORIGINALITY, RIGOUR & EXCELLENCE IN RESPIRATORY MEDICINE

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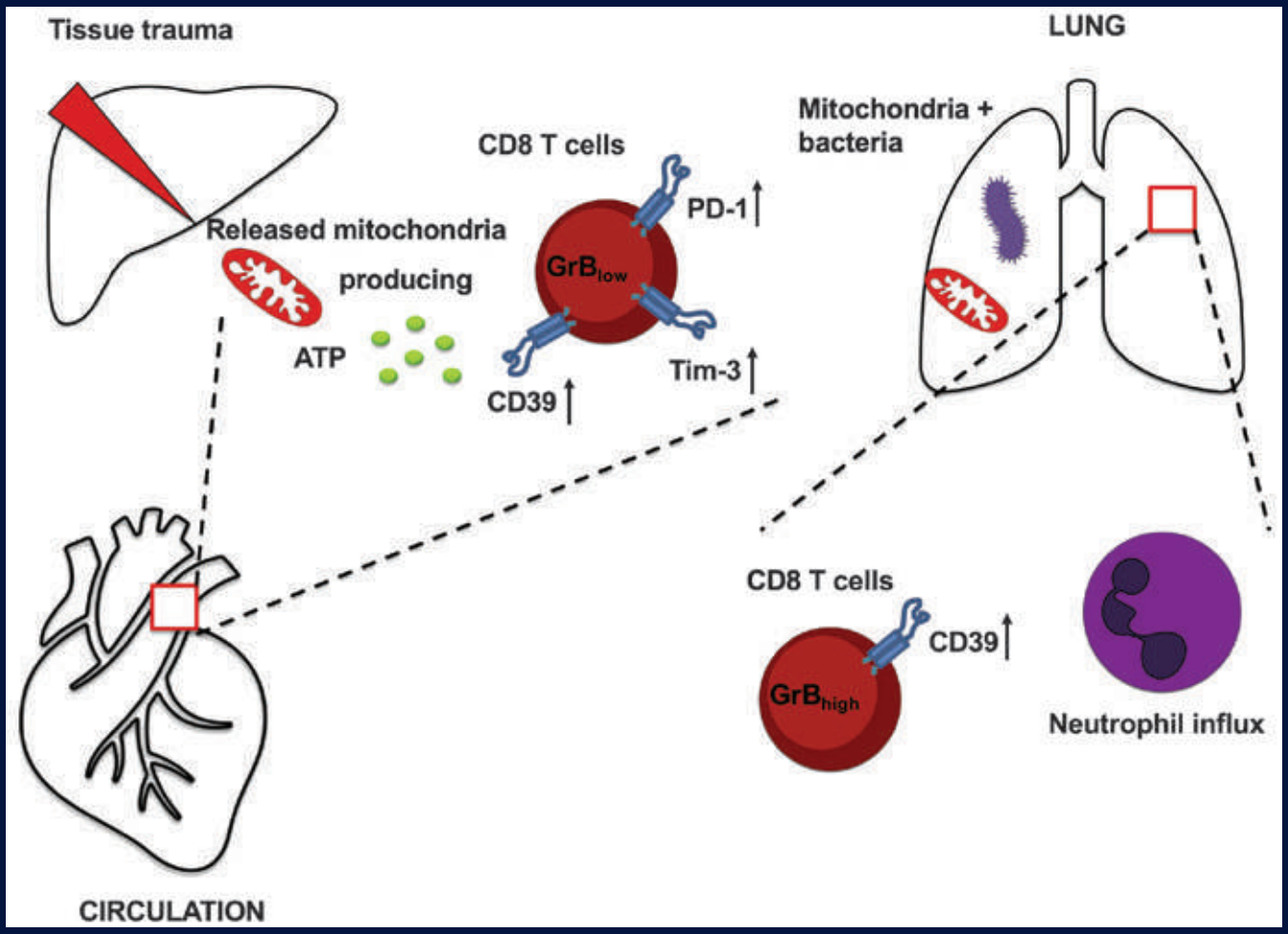

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Dá ritmo à vida[2,3*]

Para adultos com ASMA moderada a grave não controlados adequadamente com LABA/ICS.[1]

1. Zimbus® Breezhaler®, Resumo das Características do Medicamento, última atualização 12/11/2021. Disponível em https://www.ema.europa.eu/en/documents/product-information/zimbus-breezhaler-epar-product-information_pt.pdf. Consultado a 12/01/2022. Terapêutica inalada, 1x/dia, combinação de dose-fixa de acetato de indacaterol, brometo de glicopirrónio e furoato de mometasona. Mistura de lactose em pó para inalação, doses únicas, através do dispositivo BREEZHALER® que permite a confirmação de doses[1]. *Entende-se por 'dá ritmo à vida' que o doente ao tomar Zimbus® Breezhaler® poderá obter melhorias de função pulmonar, controlo de sintomas, qualidade de vida e redução de exacerbações *vs* terapêuticas padrão. **2.** Gessner C, *et al.* Fixed-dose combination of indacaterol/glycopyrronium/mometasone furoate once-daily versus salmeterol/fluticasone twice-daily plus tiotropium once-daily in patients with uncontrolled asthma: A randomised, Phase IIIb, non-inferiority study (ARGON). Respir Med. 2020 Aug-Sep;170:106021. **3.** Kerstjens HAM, *et al.* Once-daily, single-inhaler mometasone-indacaterol-glycopyrronium *versus* mometasone-indacaterol or twice-daily fluticasone-salmeterol in patients with inadequately controlled asthma (IRIDIUM): a randomised, double-blind, controlled phase 3 study. Lancet Respir Med. 2020 Oct;8(10):1000-1012. **4. EPAR:** European public assessement report. https://www.ema.europa.eu/en/documents/assessment-report/zimbus-breezhaler-epar-public-assessment-report_en.pdf; consultado em 21/05/2021. **LABA**: Agonista Beta2 de longa duração de ação; **LAMA:** antagonista muscarínico de longa duração de ação; **ICS**: corticosteroides inalados.

Zimbus Breezhaler 114 microgramas/46 microgramas/136 microgramas pó para inalação, cápsulas
NOTA: Antes de prescrever consulte o RCM do medicamento. APRESENTAÇÃO: Pó para inalação, cápsula (pó para inalação). Cada cápsula contém 150 microgramas de indacaterol, 50 microgramas de glicopirrónio e 160 microgramas de furoato de mometasona. Cada dose libertada (a dose libertada através do aplicador bocal do inalador) contém 114 microgramas de indacaterol (na forma de acetato), 8 microgramas de brometo de glicopirrónio equivalente a 46 microgramas de glicopirrónio e 136 microgramas de furoato de mometasona. Excipiente(s) com efeito conhecido: Cada cápsula contém 25 mg de lactose mono-hidratada. **INDICAÇÕES:** Zimbus Breezhaler está indicado como terapêutica de manutenção da asma em doentes adultos não controlados adequadamente com uma associação de um agonista $beta_2$ de ação prolongada e uma dose alta de corticosteroide inalado em regime de manutenção que experimentaram uma ou mais exacerbações da asma no ano anterior. **POSOLOGIA: Adultos:** A dose recomendada é uma cápsula inalada uma vez por dia. A dose máxima recomendada é de 114 µg/46 µg/136 µg uma vez por dia. **Doentes pediátricos (< 18 anos):** Não recomendado em doentes com menos de 18 anos de idade. **Populações especiais:** ***População idosa:*** Não é necessário ajuste de dose em doentes idosos (65 anos de idade ou mais). ***Compromisso renal:*** Não é necessário ajuste de dose em doentes com compromisso renal ligeiro a moderado. Deve ter-se precaução em doentes com compromisso renal grave ou doença renal terminal que necessitem de diálise. ***Compromisso hepático:*** Não é necessário ajuste de dose em doentes com compromisso hepático ligeiro a moderado. Não existem dados disponíveis sobre a utilização deste medicamento em doentes com compromisso hepático grave, como tal deve ser utilizado nestes doentes apenas se o benefício esperado superar o risco potencial. **Modo de administração:** Apenas para utilização por via inalatória. As cápsulas não podem ser engolidas. Os doentes que não sintam melhorias na sua respiração devem ser questionados se estão a engolir o medicamento em vez de o inalar. As cápsulas têm de ser administradas usando apenas o inalador Zimbus Breezhaler. O tratamento deve ser administrado à mesma hora do dia todos os dias. Pode ser administrado independentemente da hora do dia. Após a inalação, os doentes devem lavar a boca com água sem engolir. Se for omitida uma dose, esta deve ser tomada assim que possível. Os doentes devem ser instruídos a não tomarem mais do que uma dose por dia. As cápsulas de Zimbus Breezhaler devem ser sempre conservadas no blister para proteger da luz e humidade, e só podem ser removidas do blister imediatamente antes da utilização. **CONTRAINDICAÇÕES:** Hipersensibilidade às substâncias ativas ou a qualquer um dos excipientes. **ADVERTÊNCIAS/PRECAUÇÕES:** ♦ **Asma Aguda:** não deve ser utilizado para tratar sintomas agudos de asma, incluindo episódios agudos de broncospasmo, para os quais é necessário um broncodilatador de curta ação. ♦ **Hipersensibilidade:** Se ocorrer uma reação de hipersensibilidade, Zimbus Breezhaler deve ser descontinuado imediatamente e deverá ser instituída terapêutica alternativa. ♦ **Broncospasmo paradoxal**: Tal como com outra terapêutica inalatória, a administração deste medicamento pode resultar em broncospasmo paradoxal, o qual pode colocar a vida em risco. Se tal ocorrer, o tratamento deve ser interrompido imediatamente e deverá ser instituída terapêutica alternativa. ♦ **Efeitos cardiovasculare**s: Tal como outros medicamentos contendo agonistas adrenérgicos $beta_2$, este medicamento pode produzir um efeito cardiovascular clinicamente significativo em alguns doentes conforme avaliado através de aumentos na frequência cardíaca, pressão arterial e/ou sintomas, alterações do electrocardiograma (ECG). Este medicamento deve ser usado com precaução em doentes com patologias cardiovasculares (doença arterial coronária, enfarte agudo do miocárdio, arritmias cardíacas, hipertensão, suspeita ou confirmação de prolongamento do intervalo QT, distúrbios convulsivos ou tirotoxicose e em doentes com resposta aumentada aos agonistas adrenérgicos $beta_2$. ♦ **Hipocaliemia:** Os agonistas adrenérgicos $beta_2$ podem causar uma hipocaliemia significativa em alguns doentes, o que potencialmente pode levar a reações adversas cardiovasculares. Em doentes com asma grave, a hipocaliemia pode ser potenciada pela hipoxia e pela terapêutica concomitante, o que pode aumentar a suscetibilidade a arritmias cardíacas. ♦ **Hiperglicemia:** A inalação de doses elevadas de agonistas adrenérgicos $beta_2$ e corticosteroides pode produzir um aumento da glucose plasmática. Nos doentes diabéticos, ao iniciar o tratamento, a glucose plasmática deve ser monitorizada mais cuidadosamente. ♦ **Efeitos anticolinérgicos:** Tal como com outros medicamentos anticolinérgicos, este medicamento deve ser utilizado com precaução em doentes com glaucoma de ângulo fechado ou retenção urinária. ♦ **Doentes com compromisso renal grave:** Nos doentes com compromisso renal grave (taxa de filtração glomerular estimada inferior a 30 ml/min/1,73 m^2), incluindo aqueles com doença renal terminal que necessitam de diálise, deve ser utilizado apenas se o benefício esperado for superior ao risco potencial. ♦ **Prevenção de infeções orofaríngeas:** De modo a reduzir o risco de infeção orofaríngea por *candida albicans*, os doentes devem ser aconselhados a lavar a boca ou a gargarejar com água, sem a engolir, ou a lavar os dentes após inalarem a dose prescrita. ♦ **Efeitos sistémicos dos corticosteroides:** Podem ocorrer efeitos sistémicos dos corticosteroides inalados, principalmente no caso de doses elevadas prescritas por períodos prolongados. Os efeitos sistémicos possíveis podem incluir: síndrome de Cushing, manifestações Cushingóides, supressão adrenal, atraso do crescimento em crianças e adolescentes, diminuição da densidade mineral óssea, cataratas, glaucoma e, mais raramente, uma série de efeitos psicológicos ou comportamentais incluindo hiperatividade psicomotora, distúrbios do sono, ansiedade, depressão ou agressão (especialmente em crianças). Como tal, é importante que a dose de corticosteroide inalado seja titulada para a dose mais baixa na qual o controlo efetivo da asma é mantido. Podem ser notificados distúrbios visuais com o uso de corticosteroides sistémicos ou tópicos (incluindo intranasais, inalados ou intraoculares). Doentes que apresentem sintomas como visão turva ou outros distúrbios visuais, devem ser considerados para encaminhamento a um oftalmologista para avaliação de possíveis causas dessas perturbações visuais, as quais podem incluir cataratas, glaucoma ou doenças raras como a corioretinopatia central serosa (CRCS), que foram notificadas após o uso de corticosteroides sistémicos ou tópicos. Este medicamento deve ser administrado com precaução em doentes com tuberculose pulmonar ou em doentes com infeções crónicas ou não tratadas. ♦ **Gravidez:** Este medicamento deve ser utilizado durante a gravidez apenas se o benefício esperado para o doente justificar o potencial risco para o feto. ♦ **Amamentação:** Tem que ser tomada uma decisão sobre a descontinuação da amamentação ou a descontinuação/abstenção da terapêutica, tendo em conta o benefício da amamentação para a criança e o benefício da terapêutica para a mulher. ♦ **Trabalho de parto:** Como outros medicamentos contendo agonistas adrenérgicos $beta_2$, o indacaterol pode inibir o trabalho de parto devido a um efeito relaxante no músculo liso uterino. **INTERAÇÕES:** ♦ **Bloqueadores adrenérgicos beta:** este medicamento não deve ser administrado com bloqueadores adrenérgicos beta (incluindo gotas para os olhos) a menos que existam razões importantes para a sua utilização ♦ **Medicamentos conhecidos por prolongarem o intervalo QTc:** este medicamento deve ser administrado com precaução a doentes que estejam a ser tratados com inibidores da monoamina oxidase, antidepressivos tricíclicos ou medicamentos conhecidos por prolongar o intervalo QT. ♦ **Tratamento hipocaliémico:** O tratamento hipocaliémico concomitante com derivados da metilxantina, esteróides ou diuréticos não poupadores de potássio pode potenciar o possível efeito hipocaliémico dos agonistas adrenérgicos b eta_2. ♦ **Interação com inibidores do CYP3A4 e da glicoproteína P:** A inibição do CYP3A4 e da glicoproteína P (gp-P) não tem impacto na segurança de doses terapêuticas de Zimbus Breezhaler. ♦ **Outros antimuscarínicos de ação prolongada e agonistas adrenérgicos $beta_2$ de ação prolongada:** A coadministração deste medicamento com outros medicamentos contendo antagonistas muscarínicos de ação prolongada ou agonistas adrenérgicos $beta_2$ de ação prolongada não foi estudada e não é recomendada. ♦ **Cimetidina ou outros inibidores do transporte de catiões orgânicos:** Não é esperada qualquer interação medicamentosa clinicamente relevante. **EFEITOS INDESEJÁVEIS:** ♦ **Frequentes (≥1% a <10%) e potencialmente graves:** hipersensibilidade. ♦ **Muito frequentes:** asma (exacerbação), nasofaringite. ♦ **Frequentes (≥1% a <10%):** infeção do trato respiratório superior, candidíase, infeção do trato urinário, cefaleia, taquicardia, dor orofaríngea, tosse, disfonia, gastroenterite, dor musculosquelética. espasmos musculares, pirexia. ♦ **Pouco frequentes (≥0,1% a <1%):** hiperglicemia, cataratas, boca seca, erupção cutânea, prurido, disúria. Para mais informações, consultar o titular de autorização de introdução no mercado ou o representante local do titular de autorização de introdução no mercado. Medicamento sujeito a receita médica. **Escalão de comparticipação:** Escalão B. **Titular da AIM**: Novartis Europharm Limited. **Representante local:** Laboratório Medinfar - Produtos Farmacêuticos, S.A. - Rua Henrique de Paiva Couceiro, Nº 29, Venda Nova, 2700-451 Amadora Informações Essenciais Compatíveis com o Resumo das Características do Medicamento (ZIM_RCM20210422_IEC_v2).

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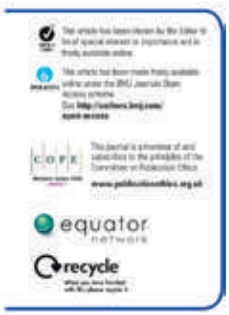

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Editorial

Este número da Edição de artigos das Revistas *Thorax* e *Archives of Disease in Childhood*, dedicados aos Sócios das Sociedades Científicas de Pneumologia, Alergologia e Imunologia Clínica, Medicina Geral e Familiar e Pediatria, tem uma série de artigos centrados na criança.

E, curiosamente, são artigos que a todos interessam, pelos temas abrangentes que aqui estão representados e que, ou têm a oportunidade da atualidade, com foco no SARS-CoV-2, ou têm expressão de indicadores ao longo do ciclo de vida.

Senão, vejamos. Na criança, sobretudo nos 2 primeiros anos de vida, a bronquiolite aguda é a doença respiratória aguda com maior prevalência, responsável por uma enorme carga de doença, que nos Países de elevados rendimentos implica uma utilização assoberbada dos serviços de saúde, desde o ambulatório, consultas e urgência, ao internamento em enfermaria e em unidades de cuidados intensivos. Mas, nos Países com rendimentos mais baixos a carga reflete-se em mortalidade infantil. O principal agente causal, quer da bronquiolite, como da Pneumonia abaixo dos 5 anos de idade é o vírus sincicial respiratório (VSR). Ora. Nos últimos 2 anos, um outro vírus recebeu um enorme protagonismo, anulando a expressão dos vírus de incidência sazonal, como o VSR e o influenza. Manteve-se o rinovírus. O artigo de Giorgio Cozzi et al, sumariza de forma objetiva o que vivemos: ausência da expressão de doença respiratória aguda no lactente associada aos responsáveis do costume e ausência, felizmente, de expressão de doença respiratória nos mesmos grupos etários associada ao SARS-CoV-2. Foram 2 invernos estranhos... mas a retoma de uma vida mais livre veio também alterar sazonalidades, gravidades e idades de incidência. Relatos que iremos ver no futuro.

Mas continuamos perplexos com as razões que levam uma criança a apresentar mais sintomas ou maior gravidade e persistência de doença em comparação com outras crianças expostas que não apresentam doença. Os 2 artigos seguintes, de Carla Rebeca Da Silva Sena et al. e Hanna Creese et al. Descrevem fatores endógenos (eNO) e exógenos (desigualdade socio-económica) como reguladores negativos e positivos para a expressão de doença respiratória na criança. Poderão, no futuro estes indicadores integrar as equações de decisão clínica e a personalização de intervenções terapêuticas?

Em todo o caso, os marcadores não invasivos de inflamação têm concentrado um enorme interesse nos últimos anos, com evidencia de gradualmente se torna mais robusta. O artigo de Simon Couillard et al volta ao marcador FeNO como fator interessante de monitorização de terapêutica.

Imersos num Mundo assimétrico e instável, chegam-nos de outras latitudes artigos relacionados com modelos diagnósticos para a tuberculose em Países de alta prevalência e preocupações, legítimas, das pessoas com doença respiratória sobre os detentores da Indústria Farmacêutica. No caso do artigo de Ruth Tal-Singer, quando as tabaqueiras se tornam detentoras de farmacêuticas de produtos respiratórios ...

Boas leituras! Boa Primavera!

Que este tempo possa ser aproveitado para reflexões e pequenas ações que contribual individual e coletivamente para um Mundo mais sustentável, mais equilibrado e mais agradável para ser habitado... por nós!

Teresa Bandeira

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Royal College of Paediatrics and Child Health
5–11 Theobalds Road
London WC1X 8SH
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E: **enquiries@rcpch.ac.uk**
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Prevalência da positividade da SARS-CoV-2 em bebés com bronquiolite: um estudo internacional multicêntrico

Giorgio Cozzi,[1] Luisa Cortellazzo Wiel,[2,3] Alessandro Amaddeo,[1] Antonio Gatto,[4] Manuela Giangreco,[1] Adi Klein-Kremer,[5] Samantha Bosis,[6] Davide Silvagni,[7] Carla Debbia,[8] Laura Nanni,[9] Sara Chiappa,[10] Marta Minute,[11] Ilaria Corsini,[12] Giuliana Morabito,[13] Anna Jolanda Gortan,[14] Marco Colombo,[15] Federico Marchetti,[16] Davide Garelli,[17] Arianna Piffer,[18] Fabio Cardinale,[19] Nitai Levy,[20] Antonietta Curatola,[21] Bojana Gojsina,[22,23] Suvradeep Basu,[3] Egidio Barbi,[1,2] Aleksandar Sovtic,[22,23] On behalf of the SARS-CoV-2 bronchiolitis study group

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For numbered affiliations see end of article.

Correspondence to
Dr Luisa Cortellazzo Wiel, University of Trieste, Trieste, Italy; luisacortellazzowiel@gmail.com

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RESUMO

Contexto A bronquiolite é a principal infeção aguda do trato respiratório em bebés durante a estação do Inverno. Desde o início da pandemia da SARS-CoV-2, foi registada uma redução do número de diagnósticos de bronquiolite.
Objetivo O presente estudo teve como objetivo descrever a incidência e as características clínicas da bronquiolite durante o inverno de 2020-2021 numa ampla coorte de crianças na Europa e em Israel e esclarecer o papel da SARS-CoV-2.
Enquadramento, pacientes, intervenções Realizámos um estudo transversal observacional multicêntrico em 23 serviços de urgência pediátrica na Europa e em Israel. Foram recolhidos dados clínicos e demográficos sobre todos os casos de bebés diagnosticados com bronquiolite entre 1 de Outubro de 2020 e 30 de Abril de 2021. Para cada doente inscrito, foram relatados testes de diagnóstico, tratamentos e resultados.
Principais medidas de resultados O principal resultado foi a prevalência de bronquiolite com SARS-CoV-2 positiva.
Resultados Trezentos e catorze bebés receberam um diagnóstico de bronquiolite durante o período de estudo. Entre 535 bebés que testaram positivo para SARS-CoV-2, 16 (3%) apresentaram bronquiolite. A idade média, a predominância do sexo masculino, o peso, a história de prematuridade e a presença de comorbilidades não diferiram entre os grupos positivo e negativo para SARS-CoV-2. O rinovírus foi o patogénio mais frequentemente envolvido, enquanto que o vírus sincicial respiratório (VSR) foi detetado num caso. A bronquiolite com SARS-CoV-2 apresentou um quadro clínico leve, com um doente a receber suplemento de oxigénio e nenhum a necessitar de internamento em unidades de cuidados intensivos pediátricos ou neonatais.
Conclusões Durante a pandemia da SARS-CoV-2, observou-se uma diminuição acentuada do número de diagnósticos de bronquiolite e o desaparecimento da epidemia de inverno do VSR. A bronquiolite relacionada com a SARS-CoV-2 foi rara e, na sua maioria, apresentou um quadro clínico leve.

Key messages

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ► Bronchiolitis is the primary cause of infants' hospitalisation and death for a viral infection in Western countries.
- ► While most cases are due to respiratory syncytial virus infections, other respiratory viruses, such as rhinovirus, adenovirus and coronavirus can be involved.
- ► To date, no data are available on the prevalence of SARS-CoV-2-related bronchiolitis.

WHAT THIS STUDY ADDS

- ► This multicentre international study shows that SARS-CoV-2 infection in infants is an uncommon cause of bronchiolitis.
- ► SARS-CoV-2-related bronchiolitis mostly displays a mild clinical course, consistent with rhinovirus-sustained forms.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ► This multicentre international study provides important insights about the natural history of the SARS-CoV-2 infection in infants and the clinical course of SARS-CoV-2-related bronchiolitis.

INTRODUCTION

Bronchiolitis is the most common acute lower respiratory tract infection in infants[1] and the leading cause of hospitalisation and death for a viral infection in Western countries.[2] Clinical features include tachypnoea, nasal flaring, chest retractions, impaired nutrition and hydration and auscultatory wheeze and crackles. Most cases are due to respiratory syncytial virus (RSV) infections. Nevertheless, other respiratory viruses, such as rhinovirus, adenovirus and coronavirus can be responsible for the development of the disease. The management of bronchiolitis is mainly supportive, including oxygenation, ventilation, nutrition and hydration, as recommended by several international guidelines.[3–5]

Data on SARS-CoV-2 infection in the paediatric age suggest that, in most cases, children develop a milder disease compared with adults, with hospitalisation or admission to intensive care unit (ICU) being required in only a minority of cases.[6] To date, little data are available about the role of SARS-CoV-2 in the development of bronchiolitis in infants and newborns.[7 8]

The aim of this study was to assess the prevalence of bronchiolitis associated with SARS-CoV-2 infection during the 2020–2021 winter season, and to describe its clinical course in comparison to bronchiolitis related to other viruses.

METHODS

A multicentre international cross-sectional study was conducted, involving 23 centres, 15 from Italy, 4 from Switzerland, 2 from Israel, 1 from the UK and 1 from Serbia.

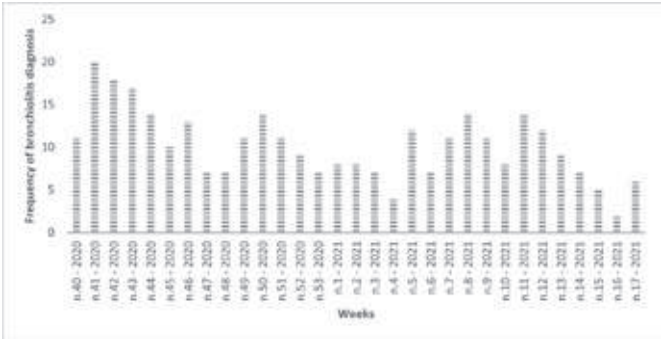

Figure 1 Distribution of the cases of bronchiolitis during the study period.

The enrolment took place from 1 October 2020 to 30 April 2021.

Eligible patients were infants diagnosed with bronchiolitis at the paediatric emergency department (PED).

Inclusion criteria were age from 0 to 12 months, clinical diagnosis of bronchiolitis following the current international guidelines[3–5] and the availability of a SARS-CoV-2 molecular test result. The disease diagnostic criteria include symptoms of upper respiratory tract infection (URTI), such as rhinorrhoea and cough, signs of respiratory distress (eg, high respiratory rate for age, use of an accessory respiratory muscle, intercostal retractions, nasal flaring, crackles or wheeze, low oxygen saturation levels, changes in skin colour), fever, exposure to subjects displaying symptoms consistent with URTI and the occurrence during the epidemic season.

Children who received a diagnosis of bronchiolitis but were older than 12 months of age were excluded from participation in the study. Patients already enrolled in the study were excluded from further participation if a second episode occurred.

Data were collected with a specific form (see online supplemental appendix) and reported in an electronic database through the Research Electronic Data Capture platform.

The following characteristics were recorded for each enrolled patient: age, sex, presence of comorbidities, diagnostic tests performed in the PED or during hospitalisation (blood tests, chest X-ray, nasal or pharyngeal swabs), use of respiratory support in the PED and during hospitalisation (oxygen supplementation, high flow nasal cannula (HFNC), continuous positive airway pressure (CPAP), non-invasive ventilation (NIV), intubation), admission status and length of hospitalisation. Moreover, every participating centre provided data regarding the overall number of attendances, the number of attending infants and the number of infants tested positive for SARS-CoV-2 at the PED during the study period. The total number of bronchiolitis diagnoses and attendances during the 2019–2020 and 2018–2019 was also collected. SARS-CoV-2 positivity was detected through a molecular test, performed through nasal or nasopharyngeal swab at the PED or during hospitalisation.

The primary study outcome was the prevalence of SARS-CoV-2 positivity among infants with bronchiolitis. The secondary outcome was the comparison of infants positive and negative for SARS-CoV-2 for the following variables: need for oxygen supplementation, NIV, intubation, feeding and hydration support, rate of hospitalisation, admission to ICU, length of hospital stay and death.

Statistical analysis

Assuming a prevalence of SARS-CoV-2-related bronchiolitis of 10% among infants attending the PED for bronchiolitis, with a precision of 5% and alpha=0.05, 139 subjects were needed to complete the study.

The characteristics of the study sample were synthesised with frequency and percentage for categorical variables and with median and IQR for continuous variables. The prevalence of SARS-CoV-2-related bronchiolitis was calculated as the ratio between the number of infants with bronchiolitis tested positive for SARS-CoV-2 attending the PED during the study period and the total number of infants with bronchiolitis attending the PED during the same time.

Differences between SARS-CoV-2-related bronchiolitis and bronchiolitis not related to SARS-CoV-2 were evaluated by the χ^2 test or the Fisher's exact test, when appropriate, for categorical variables and by the non-parametric Mann-Whitney U test for continuous variables. All analyses were conducted using SAS software, V.9.4 (SAS Institute, Cary, North Carolina, USA), and a p value <0.05 was considered statistically significant.

RESULTS

From October 2020 to April 2021, 224 119 children were evaluated at the PED of the participating centres. Among them, 19 087 were infants and 314 received a diagnosis of bronchiolitis. During the study period, 535 (3%) infants tested positive for SARS-CoV-2: among the latter, 16 (3%) received a diagnosis of bronchiolitis.

Figure 1 shows the distribution of the cases of bronchiolitis during the study period. Table 1 presents the flow of the number of attendances and diagnoses of bronchiolitis in the last three winter seasons in the participating centres. The number of PED attendances progressively declined. The diagnoses of bronchiolitis drastically dropped in the 2020–2021 winter season compared with the two previous ones. Table 2 describes the main demographical and clinical characteristics of the enrolled patients. Infants with bronchiolitis who tested positive for SARS-CoV-2 displayed clinical features consistent with those infants who tested negative for SARS-CoV-2. The median age was 4 months (IQR: 3–4) and 5 months (IQR: 2–8) in the SARS-CoV-2-positive and SARS-CoV-2-negative groups, respectively. Gender, weight, history of prematurity and presence of comorbidities did not differ between the two groups.

One patient affected by SARS-CoV-2 also proved positive for rhinovirus. Forty (13%) SARS-CoV-2-negative patients tested positive for other viruses, mostly rhinovirus and in one case RSV (table 2). Infants with SARS-CoV-2 positivity were more likely to have intrafamilial contact, with 10 (62.5%) having at least 1 parent positive for SARS-CoV-2. Nine infants (3.0%) in the SARS-CoV-2-negative group had a SARS-CoV-2 familial contact. Among these, three tested positive for other viruses, namely two for rhinovirus and one for rhinovirus and RSV.

Table 3 shows the diagnostic tests performed, the treatments received and the outcome of the population. Feeding support and hydration were provided to 2 (12.5%) infants positive for SARS-CoV-2 and 63 (21.1%) infants negative for SARS-CoV-2 (p=0.54). Oxygen supplementation was required by 1 (6.3%) patient in the SARS-CoV-2-positive group and 81 (27.2%) in the SARS-CoV-2-negative group (p=0.08). The same patient in the SARS-CoV-2-positive group received HFNC, whereas among the infants who tested negative for SARS-CoV-2, 35 (12%) received HFNC, 4 (1%) CPAP, 1 (0.3%) NIV.

None of the infants in the SARS-CoV-2-positive group required admission to neonatal ICU (NICU) or paediatric ICU (PICU), eight (50.0%) were admitted to the paediatric ward and four (25.0%) were discharged from the PED. Among these subjects, the length of hospitalisation was similar between patients who tested positive and negative for SARS-CoV-2 (p=0.8). No infants with a diagnosis of bronchiolitis died during the study period.

Table 1 Distribution of PED attendances and bronchiolitis diagnoses by period of evaluation

Period	Number of PED attendances	Percentage reduction of PED attendances compared with the previous year	Number of bronchiolitis diagnoses	Percentage reduction of bronchiolitis diagnoses compared with the previous year
1 October 2018–30 April 2019	376 229	–	4459	–
1 October 2019–30 April 2020	311 750	17%	3988	11%
1 October 2020–30 April 2021	224 199	28%	314	92%

PED, paediatric emergency department.

Table 2 Main demographical and clinical features of infants with bronchiolitis

	SARS-CoV-2-positive bronchiolitis (n=16)	SARS-CoV-2-negative bronchiolitis (n=298)	P value
Age in months, median (IQR)	4 (3–4)	5 (2–8)	0.18
Male sex, n (%)	11 (68.7)	193 (64.8)	0.75
Weight in kg, median (IQR)	6.2 (5–7.3)	6.5 (4.7–8.2)	0.40
History of prematurity, n (%)	2 (12.5)	47 (15.8)	1.0*
Presence of at least one comorbidity, n (%)	1 (6.3)	45 (15.1)	0.48*
Chronic pulmonary disease	1 (6.2)	18 (6.0)	1.0*
Congenital heart disease	0 (0.0)	17 (5.7)	1.0*
Genetic syndrome	0 (0.0)	11 (3.7)	1.0*
Other	0 (0.0)	22 (7.4)	0.61*
Positivity for at least one other virus, n (%)	1 (6.3)	40 (13.4)	0.62*
Rhinovirus	1 (6.3)	27 (9.0)	
Adenovirus	0	6 (2.0)	
Parainfluenza virus	0	4 (1.3)	
Coronavirus NL63	0	3 (1.0)	
Metapneumovirus	0	2 (0.7)	
Respiratory syncytial virus	0	1 (0.3)	
Bocavirus	0	1 (0.3)	
Enterovirus	0	3 (1.0)	
SARS-CoV-2-positive parents, n (%)			
Both parents	7 (43.8)	3 (1.0)	<0.0001*
Father	0 (0.0)	1 (0.3)	
Mother	3 (18.7)	5 (1.7)	

*Fisher's exact test.

Table 3 Diagnostics tests, treatments performed and outcomes of infants with bronchiolitis

	SARS-CoV-2-positive bronchiolitis (n=16)	SARS-CoV-2-negative bronchiolitis (n=298)	P value
Diagnostic tests, n (%)			
Blood tests	8 (50.0)	136 (45.6)	0.73
Chest X-ray	4 (25.0)	95 (31.9)	0.78*
Chest CT scan	0 (0.0)	2 (0.7)	1.0*
Treatment received, n (%)			
Hydration	2 (12.5)	63 (21.1)	0.54*
Oxygen supplementation	1 (6.3)	81 (27.2)	0.08*
Any non-invasive respiratory support	1 (6.3)	36 (12.1)	0.70*
HFNC	1 (6.3)	35 (11.7)	1.0*
CPAP	0 (0.0)	4 (1.3)	1.0*
NIV	0 (0.0)	1 (0.3)	1.0*
Mechanical ventilation, n (%)	0 (0.0)	0 (0.0)	–
Pharmacological therapies, n (%)			
Inhaled steroids	0 (0.0)	28 (9.4)	0.38*
Inhaled epinephrine	1 (6.3)	26 (8.7)	1.0*
Inhaled albuterol	3 (18.7)	135 (45.3)	0.04*
Inhaled hypertonic solution	0 (0.0)	25 (8.4)	0.63*
Systemic steroids	1 (6.3)	71 (23.8)	0.13*
Antibiotic	3 (18.7)	64 (21.5)	1.0*
Outcome, n (%)			
Discharge from the PED	4 (25.0)	147 (49.3)	0.07*
Short observation in the PED	4 (25.0)	57 (19.1)	0.53*
Admission to the paediatric ward	8 (50.0)	110 (36.9)	0.29
Admission to the NICU	0 (0.0)	4 (1.3)	1.0*
Admission to the PICU	0 (0.0)	16 (4.4)	1.0*
Death	0 (0.0)	0 (0.0)	–
Days of hospitalisation, median (IQR)	3 (2–4)	3 (2–5)	0.80

*Fisher's exact test.
CPAP, continuous positive airway pressure; HFNC, high flow nasal cannula; NICU, neonatal intensive care unit; NIV, non-invasive ventilation; PED, paediatric emergency department; PICU, paediatric intensive care unit.

DISCUSSION

This large multicentre international study showed a low prevalence of SARS-CoV-2-related bronchiolitis, with only 16 (5%) out of 314 infants with bronchiolitis testing positive for SARS-CoV-2. Moreover, among 535 infants who tested positive for SARS-CoV-2, only 16 (3%) had a diagnosis of bronchiolitis.

SARS-CoV-2-related clinical pictures in adults range from URTI to severe pneumonia and acute respiratory distress syndrome, the latter constituting the leading cause of morbidity and mortality from SARS-CoV-2 worldwide.[9] Despite initial concerns, SARS-CoV-2 infection in children proved to have a mild course in most cases,[10–13] with only mild respiratory symptoms.[14] To date, only few cases of SARS-CoV-2-related bronchiolitis have been described.[7 8 15] In this study, we reported the largest cohort of this disease, confirming the low prevalence of bronchiolitis in infants who tested positive for SARS-CoV-2. These data provide important highlights about the natural history of the SARS-CoV-2 infection in infants, which had been unknown so far.

Consistent with previous data, we found that the clinical characteristics of infants with bronchiolitis positive for SARS-CoV-2 did not differ from those negative for SARS-CoV-2. The median age was 4 months, there was a male sex predominance and 3 out of 16 infants had a history of prematurity. Only one patient had a pre-existing chronic pulmonary condition.

Our data showed a relatively mild course of SARS-CoV-2 bronchiolitis. No infants required NICU or PICU admission, half needed hospitalisation with only one patient requiring non-invasive respiratory support (HFNC). Only 2 patients (13%) in the SARS-CoV-2-positive group needed hydration compared with 63 (21%) in the SARS-CoV-2-negative group, and only 1 patient with SARS-CoV-2 bronchiolitis required oxygen supplementation. These data support the observation that SARS-CoV-2-related bronchiolitis has a relatively mild course. Only one child had a co-infection, testing positive for both SARS-CoV-2 and rhinovirus, without displaying a worse clinical course.

In accordance with previous data,[16 17] during the study period, a marked decrease in PED attendances was registered compared with the 2018–2019 (−40%) and 2019–2020 (−17%) winter season. Remarkably, the reduction of bronchiolitis diagnoses was even higher, with a 93% and 92% decrease in the 2020–2021 winter season compared with 2018–2019 and 2019–2020, respectively. These data confirm that the SARS-CoV-2 pandemic deeply impacted the epidemiology of respiratory infections in infants and children, as a result of the significant reduction of the circulation of viruses, and in particular of RSV.[18] Several factors can be involved in this epidemiological change: public health measures such as social distancing, travel restrictions, compulsory use of face masks and hand sanitisation, likely reduced exposure and suppressed interhuman transmission of SARS-CoV-2 and other viruses. Rhinovirus was the primary pathogen isolated in our series, as already reported in other studies performed during the SARS-CoV-2 pandemic.[19 20] The observed frequency of rhinovirus is consistent with the average circulation of the virus, which has its peak during early fall, and to a lesser extent, during spring.[21 22] It has been shown that while face masks can prevent transmission of human coronaviruses and influenza viruses, they do not reduce

the transmission of droplets and aerosols containing rhinoviruses.[23] Moreover, rhinovirus persists up to 4 days on inanimate surfaces, is highly resistant to ethanol-based disinfectants[24] and reaches airways through contact with contaminated hands, on which it can persist for several hours.[25] More interestingly, we observed an almost complete absence of the RSV epidemic during the last winter in Europe and Israel, as already described in other continents.[26 27] This precluded the possibility to compare the clinical course of SARS-CoV2 bronchiolitis and RSV bronchiolitis.

Treatment of bronchiolitis is mainly supportive, and the current international guidelines recommend against the use of most pharmacological therapies[28]; however, we observed a high use of inhaled therapies (albuterol, epinephrine and hypertonic saline) and systemic steroids. Since it was beyond the scope of this study, we did not collect data about local practices and guidelines that may influence the choice of therapies. Nevertheless, in this series, infants with bronchiolitis tested positive for SARS-CoV-2 did not receive more diagnostic tests and pharmacological therapies compared with infants negative for SARS-CoV-2.

Interestingly, nine patients had a history of familial contact but tested negative for SARS-CoV-2. These patients might have been false negative for SARS-CoV-2 infection, but four of them had a rhinovirus infection and two had co-infection with enterovirus and RSV, which can explain their clinical presentation.

Our study has some limitations. First, we collected data from countries that applied different social distancing measures during the same period, thus potentially affecting the homogeneity of the results. However, we did not observe significant differences between the participating centres, neither in the reported prevalence of bronchiolitis nor in the total number of infants who tested positive for SARS-CoV-2. Second, the enrolment was limited to patients with bronchiolitis who underwent a SARS-CoV-2 test, and we were unable to assess the number of children, who, despite a clinical diagnosis of bronchiolitis, did not undergo this investigation. Nevertheless, during the study period, all infants attending the PED with respiratory symptoms were tested for SARS-CoV-2 infection, in all the participating centres except one: in the latter, the swab was performed in all infants with bronchiolitis requiring hospital observation or admission. While reporting a low prevalence of bronchiolitis during the winter season, we could have missed a delayed spread of bronchiolitis, as already reported in Australia and France,[29 30] as our data have been collected until April 2021. However, we did not observe a late raise in the number of bronchiolitis diagnoses in the last weeks of the study period. Virus detection through nasal and nasopharyngeal swabs was performed according to local practice and no common diagnostic panel was used. Thus, the exact prevalence of the different viruses may have been influenced by the sensitivity of the tests used. SARS-CoV-2-related bronchiolitis was rare, and results about the clinical course of these patients were difficult to generalise. Nevertheless, the low number of SARS-CoV-2 bronchiolitis in our cohort of >500 infants (only 3% of infants with a SARS-CoV-2 positivity) demonstrated that pulmonary involvement in infants was rare and not as severe as initially expected. Finally, the design of this study did not include a follow-up of the enrolled infants with bronchiolitis, so we were unable to assess the occurrence of further respiratory symptoms in patients with SARS-CoV-2-related bronchiolitis compared with patients with RSV or rhinovirus infections. Future studies should address this issue.

In conclusion, in this large multicentre study, analysing >19 000 infants attending the PED, we observed a low prevalence of bronchiolitis during the 2020–2021 winter season. Only 16 out of 314 infants with bronchiolitis tested positive for SARS-CoV-2, and only 3% of infants positive for SARS-CoV-2 developed bronchiolitis. Moreover, SARS-CoV-2-related bronchiolitis had a mild clinical course compared with seasonal viral bronchiolitis. Rhinovirus was the most common pathogen, while RSV was detected only in one case. Following the identification of new variants, including Omicron, in addition to the easing of restrictions, there has been a raise in the number of infants and children testing positive for SARS-CoV-2. In addition, a delayed epidemic of bronchiolitis has already been reported.[29 31] Further studies are needed to compare the results of the current study with the subsequent seasons and continuous surveillance remains mandatory.

Author affiliations
[1]IRCCS Materno Infantile Burlo Garofolo, Trieste, Italy
[2]University of Trieste, Trieste, Italy
[3]Bradford Royal Infirmary, Bradford, UK
[4]Policlinico Universitario Agostino Gemelli, Roma, Italy
[5]Hillel Yaffe Medical Center, Hadera, Israel
[6]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[7]Azienda Ospedaliera Universitaria Integrata Verona, Verona, Italy
[8]Istituto Giannina Gaslini Istituto Pediatrico di Ricovero e Cura a Carattere Scientifico, Genova, Italy
[9]Azienda Ospedaliero Universitaria Meyer, Firenze, Italy
[10]Ospedale dei Bambini di Brescia, Brescia, Italy
[11]Ospedale Regionale Ca Foncello Treviso, Treviso, Italy
[12]University Hospital of Bologna Sant'Orsola-Malpighi Polyclinic, Bologna, Italy
[13]Ospedale Santa Maria degli Angeli di Pordenone, Pordenone, Italy
[14]Azienda sanitaria universitaria Friuli Centrale, Udine, Italy
[15]ASST Sette Laghi, Varese, Italy
[16]Ospedale Santa Maria delle Croci, Ravenna, Italy
[17]Regina Margherita Children's Hospital, Turin, Italy
[18]Regional Hospital of Bellinzona and Valli, Bellinzona, Switzerland
[19]Ospedale Pediatrico Giovanni XXIII, Bari, Italy
[20]Ruth Rappaport Children's Hospital, Haifa, Israel
[21]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma, Italy
[22]Institute for Health Protection of Mother and Child of Serbia 'Dr Vukan Cupic', Beograd, Serbia
[23]University of Belgrade Faculty of Medicine, Beograd, Serbia
Twitter Laura Nanni @Lanannidoc

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Collaborators Itai Shavit, Pediatric Emergency Department, Ruth Rappaport Children's Hospital, Rambam Health Care Campus, Haifa, Israel. Federica Vanoni, Clinic of Pediatrics, Pediatric Institute of Southern Switzerland, EOC, Bellinzona, Switzerland. Alessandra Iacono, Department of Pediatrics, Santa Maria delle Croci Hospital, Ravenna, Italy. Stefano Masi, Pronto Soccorso Pediatrico, Azienda Ospedaliero Universitaria Meyer, Firenze. Emanuela Piccotti, Pronto Soccorso Pediatrico, IRCCS pediatrico Gaslini, Genova. Anna Maria Plebani, Pronto Soccorso Pediatrico, Ospedale Filippo del Ponte, ASST Sette Laghi, Varese, Italy. Valentina Tranchino, Department of Pediatrics and Emergency, Pediatric Hospital Giovanni XXIII, Bari, University of Bari, Italy. Gregorio Paolo Milani, Pediatric Unit, Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico, Milano, Lombardia, Italy; Department of Clinical Science and Community Health, Università degli Studi di Milano, Milan, Lombardia, Italy. Sara Pedicini, Clinica Pediatrica, Azienda Sanitaria Universitaria Integrata Friuli Centrale (ASUFC). Vered Nir, Hillel Yaffe Medical Center, Haifa, Israel. Jelena Visekruna, Mother and Child Health Institute of Serbia and School of Medicine University of Belgrade.
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ORCID iDs
Luisa Cortellazzo Wiel http://orcid.org/0000-0003-0069-560X
Alessandro Amaddeo http://orcid.org/0000-0003-2117-0781
Antonio Gatto http://orcid.org/0000-0002-8778-9328
Nitai Levy http://orcid.org/0000-0003-2476-9204

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DPOC, doença pulmonar obstrutiva crónica

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Informações essenciais compatíveis com o Resumo das Características do Medicamento

Bevespi Aerosphere 7,2 microgramas/5 microgramas, suspensão pressurizada para inalação. Cada dose única (dose libertada que sai do aplicador bucal) contém 9 microgramas de brometo de glicopirrónio, equivalente a 7,2 microgramas de glicopirrónio, e 5 microgramas de fumarato de formoterol di-hidratado. Isto corresponde a uma dose calibrada de 10,4 microgramas de brometo de glicopirrónio, equivalente a 8,3 microgramas de glicopirrónio, e 5,8 microgramas de fumarato de formoterol di-hidratado. **Indicações terapêuticas:** Bevespi Aerosphere é indicado como tratamento broncodilatador de manutenção para o alívio de sintomas em doentes adultos com doença pulmonar obstrutiva crónica (DPOC). **Posologia e modo de administração:** Posologia: A dose recomendada é duas inalações duas vezes por dia (duas inalações de manhã e duas inalações à noite). Os doentes devem ser aconselhados a não fazer mais de 2 inalações duas vezes por dia. Se for omitida uma dose, esta deve ser tomada assim que for possível e a dose seguinte deve ser tomada no horário habitual. Não deve tomar-se uma dose a dobrar para compensar uma dose esquecida. Populações especiais: *Idosos*: Não é necessário qualquer ajuste de dose em doentes idosos. *Compromisso renal*: Bevespi Aerosphere pode ser utilizado na dose recomendada em doentes com compromisso renal ligeiro a moderado. Em doentes com compromisso renal grave ou com doença renal em fase terminal com necessidade de diálise, só deve ser utilizado se o benefício esperado for superior ao risco potencial. *Compromisso hepático*: Bevespi Aerosphere pode ser utilizado na dose recomendada em doentes com compromisso hepático ligeiro a moderado. Não existem dados relevantes sobre a utilização de Bevespi Aerosphere em doentes com compromisso hepático grave e o medicamento deve ser utilizado com precaução nestes doentes. *População pediátrica*: Não existe utilização relevante de Bevespi Aerosphere em crianças e adolescentes (com idade inferior a 18 anos) para a indicação de DPOC. Modo de administração: Utilização por via inalatória. *Instruções de utilização*: Ao acionar Bevespi Aerosphere, um volume de suspensão é expelido do recipiente pressurizado a alta velocidade. Quando o doente inala através do aplicador bucal ao mesmo tempo que aciona o inalador, a substância segue o ar inspirado para as vias respiratórias. Os doentes devem ser instruídos sobre a técnica de inalação correta. É importante instruir o doente para: Ler com atenção as instruções de utilização no folheto informativo, que se encontra dentro da embalagem de cada inalador. Não utilizar o inalador se o agente exsicante, contido na bolsa de alumínio, tiver vazado da sua embalagem. Preparar o inalador agitando-o e acionando-o quatro vezes para o ar antes da primeira utilização ou duas vezes quando o inalador não tenha sido utilizado há mais de sete dias, tenha sido exposto a temperaturas baixas ou tenha caído. Para obter uma deposição pulmonar adequada das substâncias ativas, o carregamento da dose tem de ser coordenado com a inalação. Doentes com dificuldade em coordenar o carregamento da dose com a inspiração podem utilizar Bevespi Aerosphere com uma câmara expansora para garantir a administração adequada do produto. Foi demonstrada compatibilidade com a câmara expansora Aerochamber Plus Flow-Vu. **Contraindicações:** Hipersensibilidade às substâncias ativas ou a qualquer um dos excipientes. **Advertências e precauções especiais de utilização:** *Asma*: Bevespi Aerosphere não deve ser utilizado no tratamento da asma. Broncospasmo paradoxal: Se ocorrer broncospasmo paradoxal, o tratamento com o medicamento deve ser interrompido e devem ser considerados outros tratamentos. Não indicado para utilização em situações agudas: Bevespi Aerosphere não é indicado para o tratamento de episódios agudos de broncospasmo, isto é, como terapêutica de alívio. Efeitos cardiovasculares: Podem ser observados efeitos cardiovasculares, após a administração de antagonistas dos recetores muscarínicos e simpaticomiméticos, incluindo glicopirrónio ou formoterol. Bevespi Aerosphere deve ser utilizado com precaução em doentes com doenças cardiovasculares graves, tais como cardiopatia isquémica, taquiarritmias ou insuficiência cardíaca grave. Recomenda-se também precaução em doentes com tirotoxicose ou com prolongamento do intervalo QTc conhecido ou suspeito. Hipocaliemia: Os agonistas β_2-adrenérgicos podem causar hipocaliemia significativa, o que pode aumentar a suscetibilidade para arritmias cardíacas. A diminuição do potássio sérico é normalmente transitória, não necessitando de suplementação. Em doentes com DPOC grave, a hipocaliemia pode ser potenciada por hipoxia e tratamento concomitante. Hiperglicemia: A inalação de doses elevadas de agonistas β_2-adrenérgicos pode provocar o aumento da glucose plasmática. Atividade anticolinérgica: Devido à sua atividade anticolinérgica, Bevespi Aerosphere deve ser utilizado com precaução em doentes com hiperplasia da próstata sintomática, retenção urinária ou glaucoma de ângulo fechado. Compromisso renal: Como o glicopirrónio é excretado predominantemente por via renal, os doentes com compromisso renal grave (depuração de creatinina <30 ml/min), incluindo aqueles com doença renal em fase terminal com necessidade de diálise, apenas devem ser tratados com Bevespi Aerosphere se o benefício esperado superar o risco potencial. Compromisso hepático: Em doentes com compromisso hepático grave, Bevespi Aerosphere só deve ser utilizado se o benefício esperado superar o risco potencial. Estes doentes devem ser monitorizados para potenciais reações adversas. **Interações medicamentosas e outras formas de interação:** Interações farmacocinéticas: O potencial para interações metabólicas é considerado baixo. Como o glicopirrónio é eliminado principalmente por via renal, podem ocorrer potencialmente interações medicamentosas com medicamentos que afetam os mecanismos de excreção renal. Interações farmacodinâmicas: Outros antimuscarínicos e simpaticomiméticos: A coadministração de Bevespi Aerosphere com outros medicamentos contendo anticolinérgicos e/ou agonistas β_2-adrenérgicos de longa duração de ação não foi estudada e não é recomendada uma vez que poderá potenciar as reações adversas conhecidas dos antagonistas muscarínicos ou dos agonistas β_2-adrenérgicos inalados. Não existe evidência clínica de interações quando utilizado concomitantemente com outros medicamentos para a DPOC, incluindo broncodilatadores β_2-adrenérgicos de curta duração de ação, metilxantinas e esteroides orais e inalados. *Hipocaliemia induzida por fármacos*: O tratamento concomitante com derivados da metilxantina, esteroides ou diuréticos não poupadores de potássio pode potenciar o possível efeito hipocaliemico inicial dos agonistas β_2-adrenérgicos, pelo que se recomenda precaução na sua utilização concomitante. Bloqueadores β_2-adrenérgicos: Os bloqueadores β_2-adrenérgicos (incluindo colírios) podem atenuar ou inibir o efeito dos agonistas β_2-adrenérgicos, tal como o formoterol. O uso simultâneo de bloqueadores β_2-adrenérgicos não seletivos ou seletivos deve ser evitado, a não ser que existam razões determinantes para a sua utilização. Se forem necessários bloqueadores β_2-adrenérgicos (incluindo colírios), dá-se preferência a bloqueadores β_2-adrenérgicos cardiosseletivos, embora também estes devam ser administrados com precaução. *Outras interações farmacodinâmicas*: Bevespi Aerosphere deve ser administrado com precaução em doentes que estejam a ser tratados com medicamentos conhecidos por prolongar o intervalo QTc. **Fertilidade, gravidez e aleitamento:** Gravidez: Não existem dados sobre a utilização de Bevespi Aerosphere em mulheres grávidas. Bevespi Aerosphere só deve ser utilizado durante a gravidez se os benefícios esperados superarem os potenciais riscos. Amamentação: Não é conhecido se o glicopirrónio ou o formoterol são excretados no leite humano. A administração de Bevespi Aerosphere a mulheres que estão a amamentar só deve ser considerada se o benefício esperado para a mãe for superior a qualquer possível risco para o bebé. Fertilidade: Considera-se pouco provável que Bevespi Aerosphere, administrado na dose recomendada, afete a fertilidade no ser humano. **Efeitos indesejáveis:** *Frequentes*: Ansiedade, Cefaleia, Tonturas, Boca seca, Náuseas, Espasmos musculares, Infeção do trato urinário, Dor torácica. *Pouco frequentes*: Reações de hipersensibilidade, incluindo erupção cutânea e prurido, Hiperglicemia, Agitação, Irrequietude, Insónia, Tremor, Taquicardia, Palpitações, Arritmias cardíacas (fibrilhação

auricular, taquicardia supraventricular e extra-sístoles), Retenção urinária. Pede-se aos profissionais de saúde que notifiquem quaisquer suspeitas de reações adversas através do Sítio da internet: http://www.infarmed.pt/web/infarmed/submissaoram (preferencialmente) ou através dos seguintes contactos: Direção de Gestão do Risco de Medicamentos: (Tel.: +351217987373; Linha do Medicamento: 800222444 (gratuita); E-mail: farmacovigilancia@infarmed.pt). **Titular da Autorização de Introdução no Mercado:** AstraZeneca AB, SE-151 85 Södertälje, Suécia. **Representante local do Titular da Autorização de Introdução no Mercado:** Tecnimede - Sociedade Técnico-Medicinal, S.A., Rua da Tapada Grande, n.º 2, Abrunheira, 2710-089 Sintra. Informações revistas em agosto 2022. **Para mais informações deverá contactar o representante local do Titular da Autorização de Introdução no Mercado. Medicamento sujeito a receita médica. Medicamento comparticipado pelo Escalão B (69% de comparticipação no regime geral e 84% de comparticipação no regime especial). Versão 1.0 (agosto 2022).**

Informações essenciais compatíveis com o Resumo das Características do Medicamento

Riltrava Aerosphere 5 microgramas/7,2 microgramas/160 microgramas, suspensão pressurizada para inalação. Cada dose única (dose libertada que sai do aplicador bucal) contém 5 µg de fumarato de formoterol di-hidratado, 9 µg de brometo de glicopirrónio, equivalente a 7,2 µg de glicopirrónio e 160 µg de budesonida. Isto corresponde a uma dose calibrada de 5,3 µg de fumarato de formoterol di-hidratado, 9,6 µg de brometo de glicopirrónio, equivalente a 7,7 µg de glicopirrónio e 170 µg de budesonida. **Indicações terapêuticas:** Riltrava Aerosphere é indicado como tratamento de manutenção em doentes adultos com DPOC moderada a grave, que não estão adequadamente tratados com uma associação de um corticosteroide inalado e um agonista beta2 de longa duração de ação ou uma associação de um agonista beta2 de longa duração de ação e um antagonista muscarínico de longa duração de ação. **Posologia e modo de administração:** Posologia: A dose recomendada e máxima é de 2 inalações 2x/dia (2 inalações de manhã e 2 inalações à noite). Se for omitida uma dose, esta deve ser tomada assim que for possível e a dose seguinte deve ser tomada no horário habitual. Não se deve tomar uma dose a dobrar para compensar uma dose esquecida. Populações especiais: *Idosos*: Não é necessário qualquer ajuste de dose em doentes idosos. *Compromisso renal:* Este medicamento pode ser utilizado na dose recomendada em doentes com compromisso renal ligeiro a moderado. Pode também ser utilizado na dose recomendada em doentes com compromisso renal grave ou com doença renal em fase terminal com necessidade de diálise, apenas se o benefício esperado for superior ao risco potencial. *Compromisso hepático:* Este medicamento pode ser utilizado na dose recomendada em doentes com compromisso hepático ligeiro a moderado. Pode também ser utilizado na dose recomendada em doentes com compromisso hepático grave, o medicamento deve ser utilizado apenas se o benefício esperado for superior ao risco potencial. *População pediátrica*: Não existe utilização relevante deste medicamento em crianças e adolescentes (com idade inferior a 18 anos) para a indicação da DPOC. Modo de administração: Para utilização por via inalatória. *Instruções de utilização*: De forma a assegurar uma administração correta do medicamento, o médico ou outro profissional de saúde deve demonstrar ao doente como utilizar corretamente o inalador, e deve monitorizar regularmente a técnica de inalação do doente. O doente deve ser aconselhado a ler com atenção o Folheto Informativo e seguir as instruções de utilização conforme indicado no mesmo. Ao acionar Riltrava Aerosphere, um volume de suspensão é expelido do recipiente pressurizado. Quando o doente inala através do aplicador bucal ao mesmo tempo que aciona o inalador, a substância segue o ar inspirado para as vias respiratórias. Os doentes que têm dificuldade em coordenar o acionamento com a inalação podem utilizar Riltrava Aerosphere com uma câmara expansora para garantir a administração adequada do medicamento. Pode ser utilizado com câmaras expansoras incluindo o Aerochamber Plus FlowVu. **Contraindicações:** Hipersensibilidade às substâncias ativas ou a qualquer um dos excipientes. **Advertências e precauções especiais de utilização:** Não indicado para utilização em situações agudas. Este medicamento não é indicado para o tratamento de episódios agudos de broncospasmo, isto é, como terapêutica de alívio. Broncospasmo paradoxal: A administração de formoterol/glicopirrónio/budesonida pode produzir broncospasmo paradoxal com síbilos e dispneia imediatamente após a administração e pode ser potencialmente fatal. O tratamento com este medicamento deve ser imediatamente interrompido se ocorrer broncospasmo paradoxal. O doente deve ser avaliado e instituído tratamento alternativo, se necessário. Deterioração da doença: Recomenda-se que o tratamento com este medicamento não seja interrompido abruptamente. Se os doentes não considerarem o tratamento eficaz, devem manter o tratamento, mas tem de se procurar aconselhamento médico. O aumento da utilização de broncodilatadores de alívio indica um agravamento do quadro clínico subjacente e justifica uma reavaliação da terapêutica. A deterioração rápida e progressiva nos sintomas da DPOC é potencialmente fatal e o doente deve ser submetido a avaliação médica urgente. Efeitos cardiovasculares: Podem ser observados efeitos cardiovasculares, tais como arritmias cardíacas, p.ex. fibrilhação auricular e taquicardia, após a administração de antagonistas dos recetores muscarínicos e simpaticomiméticos, incluindo glicopirrónio e formoterol. Este medicamento deve ser utilizado com precaução em doentes com doença cardiovascular grave não controlada e clinicamente significativa, tal como cardiopatia isquémica instável, enfarte agudo do miocárdio, cardiomiopatia, arritmias cardíacas, e insuficiência cardíaca grave. Recomenda-se também precaução ao tratar doentes com prolongamento do intervalo QTc (QTc> 450 milissegundos para homens ou > 470 milissegundos para mulheres), conhecido ou suspeito, quer seja congénito ou induzido por medicamentos. Efeitos sistémicos com corticosteroides: Podem ocorrer efeitos sistémicos com qualquer corticosteroide inalado, particularmente em doses elevadas prescritas por longos períodos de tempo. Estes efeitos são muito menos prováveis de ocorrer com o tratamento por inalação do que com corticosteroides orais. Os efeitos sistémicos possíveis incluem síndrome de Cushing, manifestações Cushingoides, supressão suprarrenal, diminuição da densidade mineral óssea, cataratas e glaucoma. Devem ser considerados efeitos potenciais na densidade óssea especialmente com a administração de doses elevadas durante longo período de tempo em doentes com fatores de risco coexistentes para osteoporose. Perturbações visuais: Podem ser notificadas perturbações visuais com a utilização sistémica e tópica de corticosteroides. Se um doente apresentar sintomas tais como visão turva ou outras perturbações visuais, o doente deve ser encaminhado para um oftalmologista para avaliação de possíveis causas que podem incluir cataratas, glaucoma ou doenças raras, tais como CRSC, que foram notificadas após a utilização de corticosteroides sistémicos e tópicos. Transferência de terapêutica oral: Recomenda-se atenção especial nos doentes que fazem a transição de esteroides orais, uma vez que podem continuar em risco de compromisso da função suprarrenal durante um período de tempo considerável. Os doentes que necessitam de terapêutica com doses elevadas de corticosteroides ou tratamento prolongado com a dose mais elevada recomendada de corticosteroides inalados, podem estar igualmente em risco. Estes doentes podem apresentar sinais e sintomas de insuficiência suprarrenal quando expostos a *stress* grave. Deve ser considerada cobertura adicional com corticosteroides sistémicos durante períodos de *stress* ou cirurgia eletiva. Pneumonia em doentes com DPOC: Tem sido observado um aumento na incidência de pneumonia, incluindo pneumonia que requer hospitalização, nos doentes com DPOC a receberem corticosteroides inalados. Existe alguma evidência de risco aumentado de pneumonia com o aumento da dose de esteroide, não tendo sido demonstrado de forma conclusiva nos diversos estudos. Não existe evidência clínica conclusiva para diferenças dentro da mesma classe na magnitude do risco de pneumonia entre os medicamentos contendo corticosteroides inalados. Os médicos devem continuar alerta para o possível desenvolvimento de pneumonia em doentes com DPOC pois as características clínicas de tais infeções sobrepõem-se aos sintomas das exacerbações da DPOC. Os fatores de risco para pneumonia em doentes com DPOC incluem tabagismo atual, idade avançada, IMC baixo e DPOC grave. Hipocaliemia: A hipocaliemia potencialmente grave pode resultar da terapêutica com agonistas-β_2. Estes têm o potencial de produzir acontecimentos cardiovasculares adversos. Recomenda-se precaução especial na DPOC grave, pois esse efeito pode ser potencializado pela hipoxia. A hipocaliemia também pode ser potencializada pelo tratamento concomitante com outros medicamentos que podem induzir hipocaliemia, tais como derivados de xantinas, esteroides e diuréticos. Hiperglicemia: A inalação de doses elevadas de agonistas β_2-adrenérgicos pode provocar o aumento da glucose plasmática. A glicemia deve ser monitorizada durante o tratamento de acordo com as orientações estabelecidas para doentes com diabetes. Condições coexistentes: Este medicamento dever ser utilizado com precaução em doentes com tirotoxicose. Atividade anticolinérgica: Devido à sua atividade anticolinérgica, este medicamento deve ser utilizado com precaução em doentes com hiperplasia da próstata sintomática, retenção urinária ou glaucoma de ângulo fechado. Os doentes devem ser informados sobre os sinais e sintomas do glaucoma de ângulo fechado e devem ser informados para interromper a utilização deste medicamento e contactarem o seu médico imediatamente, caso algum destes sinais ou sintomas se desenvolvam. Não é recomendada a administração concomitante deste medicamento com outros medicamentos contendo anticolinérgicos. Compromisso renal: Como o glicopirrónio é excretado predominantemente por via renal, os doentes com compromisso renal grave (depuração de creatinina <30 ml/min), incluindo aqueles com doença renal em fase terminal com necessidade de diálise, apenas devem ser tratados com este medicamento se o benefício esperado superar o risco potencial. Compromisso hepático: Em doentes com compromisso hepático grave, este medicamento só deve ser utilizado se o benefício esperado superar o risco potencial. Estes doentes devem ser monitorizados para potenciais reações adversas. **Interações medicamentosas e outras formas de interação:** Interações farmacocinéticas: Prevê-se que o tratamento em associação com inibidores potentes do CYP3A, por exemplo itraconazol, cetoconazol, inibidores de protéase do VIH e medicamentos que contêm cobicistate, aumente o risco de efeitos indesejáveis sistémicos, e deve ser evitado, a não ser que o benefício seja superior ao risco aumentado de reações adversas aos corticosteroides sistémicos, nesse caso os doentes devem ser monitorizados para reações adversas aos corticosteroides sistémicos. Este facto é de relevância clínica limitada em tratamentos de curta duração (1-2 semanas). Como o glicopirrónio é eliminado principalmente por via renal, podem ocorrer potencialmente interações medicamentosas com medicamentos que afetam os mecanismos de excreção renal. Interações farmacodinâmicas: *Outros antimuscarínicos e simpaticomiméticos*: A administração concomitante deste medicamento com outros medicamentos contendo anticolinérgicos e/ou agonistas β_2-adrenérgicos de longa duração de ação não foi estudada e não é recomendada uma vez que poderá potenciar as reações adversas conhecidas dos antagonistas muscarínicos ou dos agonistas β_2-adrenérgicos inalados. A utilização concomitante de outros medicamentos beta-adrenérgicos pode ter efeitos potencialmente aditivos; portanto é necessário precaução quando outros medicamentos beta-adrenérgicos são prescritos concomitantemente com formoterol. *Hipocaliemia induzida por* medicamentos: A hipocaliemia pode aumentar a disposição para arritmias em doentes que são tratados com glicosídeos digitálicos. *Bloqueadores β-adrenérgicos*: Os bloqueadores β-adrenérgicos (incluindo colírios) podem atenuar ou inibir o efeito do formoterol. O uso simultâneo de bloqueadores β-adrenérgicos deve ser evitado, a não ser que o benefício esperado ultrapasse o risco potencial. Se forem necessários bloqueadores β-adrenérgicos, dá-se preferência a bloqueadores β-adrenérgicos cardiosseletivos. *Outras interações farmacodinâmicas*: O tratamento concomitante com quinidina, disopiramida, procainamida, anti-histamínicos, inibidores da monoamina oxidase, antidepressivos tricíclicos e fenotiazinas pode prolongar o intervalo QT e aumentar o risco de arritmias ventriculares. Além disso, L-dopa, L-tirosina, oxitocina e álcool podem prejudicar a tolerância cardíaca aos beta2-simpaticomiméticos. O tratamento concomitante com inibidores da monoamina oxidase, incluindo medicamentos com propriedades semelhantes como furazolidona e procarbazina, pode dar origem a reações hipertensivas. Existe um risco elevado de arritmias em doentes a receberem anestesia concomitante com hidrocarbonetos halogenados. **Fertilidade, gravidez e aleitamento:** A administração deste medicamento a mulheres grávidas só deve ser considerada se o benefício esperado para a mãe justificar o potencial risco para o feto. A administração deste medicamento a mulheres que estão a amamentar só deve ser considerada se o benefício esperado para a mãe for superior a qualquer possível risco para a criança. Considera-se pouco provável que este medicamento administrado na dose recomendada, afete a fertilidade no ser humano. **Efeitos indesejáveis:** *Frequentes*: Candidíase oral; Pneumonia; Hiperglicemia; Ansiedade; Insónia; Cefaleia; Palpitações; Disfonia; Tosse; Náuseas; Espasmos musculares; Infeção do trato urinário. *Pouco frequentes*: Hipersensibilidade; Depressão; Agitação; Irrequietude; Nervosismo; Tonturas; Tremor; Angina de peito; Taquicardia; Arritmias cardíacas (fibrilhação auricular, taquicardia supraventricular e extrassístoles); Irritação da garganta; Broncospasmo; Boca seca; Equimose; Retenção urinária; Dor torácica. *Muito raros*: Sinais ou sintomas de efeitos sistémicos de glucocorticoides, por exemplo supressão suprarrenal; Comportamento anormal. *Desconhecido*: Angioedema; Visão turva; Cataratas; Glaucoma. Pede-se aos profissionais de saúde que notifiquem quaisquer suspeitas de reações adversas através do Sítio da internet: http://www.infarmed.pt/web/infarmed/submissaoram (preferencialmente) ou através dos seguintes contactos: Direção de Gestão do Risco de Medicamentos: (Tel.: +351 21 798 73 73; Linha do medicamento: 800 222 444 (gratuita); E-mail: farmacovigilancia@infarmed.pt). **Titular da Autorização de Introdução no Mercado:** AstraZeneca AB, SE-151 85 Södertälje, Suécia. **Representante local do Titular da Autorização de Introdução no Mercado**: Tecnimede - Sociedade Técnico-Medicinal, S.A., Rua da Tapada Grande, n.º 2, Abrunheira, 2710-089 Sintra. Informações revistas em abril 2022. **Para mais informações deverá contactar o representante local do Titular da Autorização de Introdução no Mercado. Medicamento sujeito a receita médica. Medicamento comparticipado pelo Escalão B (69% de comparticipação no regime geral e 84% de comparticipação no regime especial). 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tecnimede

TECNIMEDE - Sociedade Técnico-Medicinal, S.A. - Rua da Tapada Grande, n.º 2 2710-089 ABRUNHEIRA, PORTUGAL
Tel.: +351 210 414 100 | Fax: +351 219 412 157
Nif: 500 626 413

FARMACOVIGILÂNCIA 800 20 28 38 LINHA VERDE

TRIBE231A1CG/NOV/2022, revalidado anualmente

Titular de AIM e comercialização:

AstraZeneca

AstraZeneca Produtos Farmacêuticos, Lda
R. Humberto Madeira n.º 7 | Queluz Baixo |
2730-097 Barcarena | Contribuinte Nº PT 502 942 240
Capital Social: 1.500,000€ | Mat. Cons. Reg. Com.
Cascais sob o N.º 502942240
Telefone: +351 214 346 100

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ARTIGO ORIGINAL

O óxido nítrico exalado mais elevado às 6 semanas de idade está associado a menos bronquiolite e pieira nos primeiros 12 meses de idade

Carla Rebeca Da Silva Sena,[1] Ediane de Queiroz Andrade,[1] Patricia de Gouveia Belinelo,[1] Elizabeth Percival,[1] Benjamin Prangemeier,[2] Christopher O'Donoghue,[2] Sandrine Terry,[2] Tanya Burke,[2] William Gunning,[2] Vanessa E Murphy,[1] Paul D Robinson,[3] Peter D Sly,[4] Peter G Gibson,[5,6] Adam M Collison,[1] Joerg Mattes[1,7]

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For numbered affiliations see end of article.

Correspondence to
Professor Joerg Mattes, Paediatrics & Child Health, The University of Newcastle, New Lambton Heights, New South Wales, Australia; joerg.mattes@newcastle.edu.au

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RESUMO

Contexto O óxido nítrico em ar exalado (eNO) é usado como marcador de inflamação das vias aéreas induzida por resposta imunológica de tipo 2. O nosso objetivo foi investigar a associação entre a incidência de eNO e bronquiolite e os sintomas respiratórios na infância, e a sua correlação com a proteína eosinófila X (PEX).

Métodos Acompanhámos bebés com 6 semanas de idade nascidos de mães com asma durante a gravidez e medimos o eNO durante o sono natural recorrendo a um analisador quimiluminescente de resposta rápida (CLD88; EcoMedics), recolhendo pelo menos 100 respirações, interpoladas para um fluxo expiratório de 50 ml/s. O PEX normalizado para creatinina foi medido em amostras de urina (uPEX/c). Foi usado um questionário padronizado para medir os sintomas no primeiro ano de vida. As associações foram investigadas com recurso a regressão linear múltipla e a modelos de regressão de Poisson robustos.

Resultados Foram obtidos níveis de eNO em 184 bebés, dos quais 125/184 (68%) dispunham de 12 meses de dados de questionário e 51/184 (28%) dispunham de uPEX/c medido. O eNO mais elevado foi associado a menos sintomas respiratórios durante as primeiras 6 semanas de vida (n=184, ß-coeficiente: -0,49, 95% IC -0,95 a -0,04, p=0,035). O eNO foi negativamente associado ao uPEX/c (ß-coeficiente: -0,004, 95% IC -0,008 a -0,001, p=0,021). A incidência de risco de bronquiolite, pieira, constipação ou doença gripal e o uso de beta-agonistas de curta duração diminuíram significativamente em 18%-24% para cada aumento unitário de eNO ppb.

Conclusão Níveis mais elevados de eNO às 6 semanas de idade podem ser um substituto para uma resposta imunitária alterada associada a menos sintomas respiratórios no primeiro ano de vida.

Key messages

What is the key question?

► Is nitric oxide in exhaled air (eNO) at 6 weeks of age associated with adverse respiratory outcomes at 12 months of life?

What is the bottom line?

►eNO may predict bronchiolitis and wheeze incidence in the first year of life.

Why read on?

►Our results highlight a potential role of eNO as a useful research tool to identify infants with increased risk for adverse respiratory outcomes in the future. The clinical significance for outcomes beyond infancy and the mechanisms underpinning the results require further studies.

INTRODUCTION

Nitric oxide (NO) is produced by NO synthases (NOS) and people with asthma have a higher expression of the inducible (i) NOS in their airways.[1 2] In asthma, iNOS expression is thought to be induced by interleukin (IL)-4 and IL-13 in airway epithelial cells in a signal transducer and activator of transcription 6 (STAT6)-dependent manner.[3] NO in exhaled air (eNO) is widely used as a non-invasive biomarker of asthma in adults and older children for type 2 immune response-induced eosinophilic airways inflammation.[4–6] However, the transcriptional upregulation of iNOS expression and NO production is controlled by a broad range of proinflammatory and anti-inflammatory cytokines in different cell types and under different conditions.[7] They include, IL-1 beta (IL-1 β), tumour necrosis factor (TNF)-alpha, interferon (IFN) gamma and lipopolysaccharide-induced nuclear factor (NF)-kappa B, hypoxia-inducible factor and STAT-1 activation in smooth muscle cells, macrophages and neutrophils.[7]

In contrast to asthma, eNO is lower in some lung diseases that are associated with exaggerated airways inflammation, such as acute respiratory syncytial virus (RSV) bronchiolitis,[8] cystic fibrosis, and primary ciliary dyskinesia (PCD).[9] Thus, the role of eNO as a surrogate marker for airways inflammation and disease is multifaceted.

A recent systematic review found 175 studies that used eNO to monitor asthma and medication use in adults and older children.[10] eNO predicted an asthma diagnosis in children and adults, as well as responsiveness to corticosteroid therapy. However, it was also highlighted that the role of eNO measurements in infants has not been completely established.[10]

Few studies have investigated eNO in young infants and differences in eNO have been reported.[11–13]

Bronchiolitis in infancy is a common virus-induced lower respiratory tract infection involving the terminal airways[14 15] and lower eNO levels have been observed during acute disease.[8] Decreased eNO levels were also described in infants with current rhinorrhoea.[16 17]

The primary aim of this analysis was to investigate whether eNO levels measured at 6 weeks of age were associated with bronchiolitis in the first 12 months of life in infants born to mothers with asthma in pregnancy. Our second aim was to explore the relationship between eNO and urinary eosinophil protein X (EPX) which is suggested to be another non-invasive marker of eosinophilic inflammation.

METHODS

This was a prospective nested cohort study, with infants recruited from mothers with mild to moderate asthma in pregnancy who participated in a randomised controlled trial (RCT), the Breathing for Life Trial (BLT). In this RCT, 1200 pregnant women were randomised to asthma management guided by fractional inhaled NO (FeNO) versus by usual clinical care as described by Murphy *et al*.[18] This study is registered with the Australian and New Zealand Clinical Trials Registry, number ACTRN12613000202763. Asthma in pregnancy was defined as self-reported, doctor-diagnosed asthma in conjunction with current asthma symptoms or inhaled asthma medication use. If consent was given by parent(s)/guardian(s), infants were prospectively followed up in Newcastle, Australia, with measurements including eNO and lung function at 6 weeks corrected gestational age, clinical assessment, and parent-reported questionnaire at 12 months of age. Infants at 6 weeks of age were seen between May 2014 and December 2019. The inclusion criteria for infant lung function testing was no apparent major birth defects or perinatal disease that would preclude performing unsedated infant lung function. Infants born preterm were included. In an effort to avoid bias, infants must not have had a respiratory illness of any kind in the 2 weeks prior to testing. If they attended the appointment they were excluded from this analysis. The day before the appointment, a team member confirmed via telephone call that the baby was asymptomatic.

During the appointment at 6 weeks of age, parents reported if the child had any bronchiolitis episodes in the past. Parents also reported any cold or influenza, upper respiratory symptoms, snuffled or blocked nose, wheezing, or any other respiratory symptoms during the first 6 weeks of life of the child. If any of these symptoms were reported, we classified that as presence of 'respiratory symptoms before 6 weeks of age'. A urine sample was collected from the infants during the 6 weeks of age appointment. At 12 months of age, parents completed the International Study of Asthma and Allergies in Childhood core questionnaire (wheezing, cough and rhinitis and eczema modules) and additional standardised respiratory symptom questions validated for infants.[19]

Main outcomes

The main outcomes described in this study included any bronchiolitis episode reported at 12 months of age. This outcome was collected using questionnaire data: 'Has your child ever suffered from bronchiolitis?' Other questions included the following other respiratory adverse outcomes: 'Has your child had wheezing or whistling in the chest in the last 12 months?'; 'In the last 12 months, did you child has had a cold or influenza more than once?'; 'In the past 12 months, has your child had a problem with sneezing, or a runny, or blocked nose when he/she did NOT have a cold or influenza?'; 'In the last 12 months, has wheezing resulted in your child attending the emergency department?'; 'Did your child take Salbutamol, Ventolin, Bricanyl, or other blue inhaler drugs in the last 12 months?'; 'Has your child had bronchiolitis more than once?'.

In order to compare subgroups in this cohort, we presented our results according to subgroups, including: (1) children who were part of the complete cohort and had lung function available at 6 weeks of age (n=457)[20]; (2) children in whom measurement of eNO was successfully collected at 6 weeks of age (n=184); (3) children with eNO measurements at 6 weeks of age and who had questionnaire data at 12 months of age (n=125) and (4) children with eNO measurements at 6 weeks and urinary EPX (uEPX) collected at 6 weeks of age (n=51).

eNO testing

Infants were unsedated and had lung function measured during behaviourally defined quiet sleep. Tests were performed with the infants lying supine, using an infant mask (sizes 0, 0/1 and 1; Homedica AG, Huenenberg, Switzerland), according to the European Respiratory Society/ American Thoracic Society (ERS/ATS) standards of infant lung function testing,[6 21] and we corrected for the mask size dead space during analysis. eNO was measured online employing an ultrasonic flow metre (Spiroson; EcoMedics AG, Duernten, Switzerland) in combination with a rapid response chemiluminescence analyser (CLD88; EcoMedics AG, Duernten, Switzerland) in the range 0–100 ppb and an error rate of ±1 ppb. Data was collected with at least three trials, and with a coefficient of variance of less than 10% between trials. eNO mean was calculated after analysis, breath-by-breath during the third quartile of exhalation. Data were included in the analysis if at least 100 breaths were measured, and values were interpolated to a flow rate of 50 mL/s using GraphPad Prism V.7.3 for Windows (GraphPad Software, San Diego, California, USA) as described by Frey *et al*.[22 23] Contamination of eNO by ambient NO was avoided by using NO-free air for inspiration. When ambient NO exceeded 5ppb and NO free air was not available for use with testing, the results were excluded from the analysis. In addition, ambient NO was measu-red before each sampling. In the analysis of the primary aim, we only included children for whom eNO levels at 6 weeks of age and questionnaire data at 12 months of age were available.

Urinary EPX

Urine samples were collected using a bag during the 6 weeks of age visit and were frozen and stored at −80 °. uEPX levels were analysed with an ELISA immunoassay (EDN ELISA Kit, MBL). In order to minimise the influence of differences in water dilution, uEPX levels were normalised by dividing by the creatinine concentration (uEPX/c).[16] The lower limit of detection was 2.4 ng/mL with the upper limit of detection 160 ng/mL. No samples fell outside of the range of detection. All samples underwent a single freeze-thaw cycle.

Statistical analysis

Differences between infants characteristics were assessed using chi-square tests or Fisher's exact test (for categorical variables) and independent sample t-tests or Wilcoxon signed-rank test (for continuous variables). Because of skewness to the left, eNO levels were transformed to the square root (sqrt) for all analyses to normalise the data prior to regression analysis. Multivariable linear regression was used to evaluate predictors for eNO at 6 weeks of age. Multivariable linear models included: sex, prematurity, age at test, parity (defined as giving birth to a fetus with a gestational age of 24 weeks or more, whether the child was born alive or was stillborn), tobacco exposure during pregnancy, and birth weight. Collinearity between variables was verified using the test for variance inflation factor. In order to evaluate the relationship between continuous variables, correlations were evaluated using Spearman's rank correlation test. The effect size of eNO levels at 6 weeks of age on the development of bronchiolitis and respiratory symptoms at 12 months of age were calculated as unadjusted incidence rate ratio (IRR) and with adjusted IRR (aIRR) obtained using a modified Poisson regression model with a robust error variance used for binary data.[24 25] Robust error variance is used to estimate incidence risk ratio more efficiently providing a less wide CI, avoiding variance overestimation when Poisson regression is applied to binary data.[24 25] For consistency of reporting, the influence of known and potential predictors (sex, prematurity, maternal atopy, age at test, parity, tobacco exposure during pregnancy, exclusive breastfeeding, maternal asthma exacerbation episode in pregnancy, and maternal inhaled corticosteroids use during pregnancy, mother's RCT group allocation) was assessed and included in all Poisson regression models. To avoid selection bias, we compared the initial cohort with our nested study in order to investigate if there were differences between the groups. In this study, a specific sample size was not targeted, instead, a convenience sample was used. Descriptive statistics and multiple linear and Poisson regression analyses were performed using Stata SE V.15 for Windows (Stata). GraphPad Prism was used to produce graphs (V.7, GraphPad Software, San Diego, California).

RESULTS

Between May 2014 and December 2019, 457 eligible infants born to mothers participating in the BLT at the Newcastle study site attended appointments to have lung function measured at 6 weeks of age. Of these infants, 70% (320 out of 457 infants) had attempted

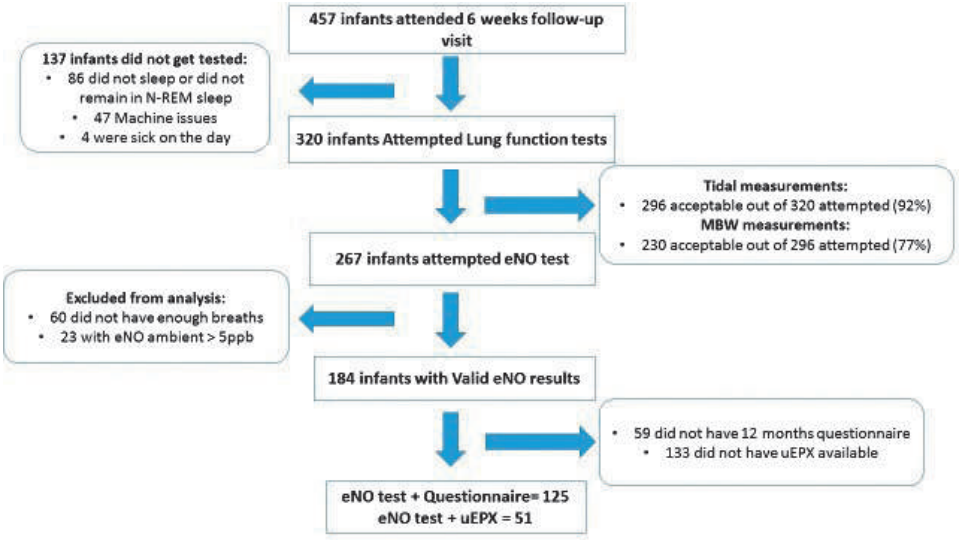

Figure 1 Flow chart showing recruitment, participation and lung function success rate. eNO, nitric oxide in exhaled air; uEPX, urinary eosinophil protein X; MBW, multiple breath washout.

lung function measurements, and 83% (267 out of 320 infants) had attempted eNO measurement. Technically acceptable eNO data (with at least 100 breaths and ambient eNO levels <5 ppb) were obtained for 69% (184 out of 267 infants). From 184 babies with eNO available, 68% (125 out of 184) had questionnaire data available at 12 months and in 28% (51 out of 184) uEPX/c was measured (figure 1). Baseline characteristics were balanced between the subgroups, with the exception of a higher proportion of male babies with available uEPX/c as a consequence of urine collection being more successful in male infants (table 1).

In the linear multivariable analysis, a significant negative association between eNO and respiratory symptoms during the first 6 weeks of life was found (table 2). eNO was also negatively associated with parity and uEPX/c (table 2). There was a significant correlation between eNO and uEPX/c (n=51, r=−0.54, p<0.001, figure 2), but no significant difference in uEPX/c levels between children with and without respiratory symptoms at 6 weeks of age (p=0.770).

We investigated the association between eNO and respiratory outcomes in the first year of life and found that eNO at 6 weeks of age was significantly higher in infants who did not have parent-reported bronchiolitis during the first 12 months of life (p=0.038). Bronchiolitis, wheeze, cold or influenza, short-acting beta-agonists use, and recurrent bronchiolitis risk incidence significantly decreased by 18% to 24% for every unit increase in transformed eNO when adjusting for other predictors and confounders (table 3). Preterm birth was positively associated with bronchiolitis, recurrent bronchiolitis and snoring incidence risk (aIRR 1.81 95% CI 1.12 to 2.92, p=0.015; aIRR 3.46, 95% CI 1.27 to 9.41, p=0.015 and aIRR 1.37, 95% CI 1.05 to 1.78, p=0.020, respectively (online supplemental table 1). Self-reported active maternal smoking during pregnancy was positively associated with wheezing in the first 12 months of life (aIRR 1.58, 95% CI 1.07 to 2.32, p=0.021), sneezing, blocked nose without cold or influenza (aIRR 1.79, 95% CI 1.08 to 2.99, p=0.025) and snoring at night without a cold (aIRR 1.43, 95% CI 1.05 to 1.94, p=0.023 in online supplemental table 1). Infants with siblings were at a higher risk of having had episodes of cold or influenza in the first year of life (aIRR 1.14, 95% CI 1.04 to 1.25, p=0.005 in online supplemental table 1). Asthma management allocation in pregnancy was not significantly associated with eNO, bronchiolitis at 12 months of age, or any other symptoms except having had a cold or influenza in the first 12 months of life (97.1% in FeNO vs 88.8% in usual care management, p=0.049).

DISCUSSION

We found that higher levels of eNO at 6 weeks of age were associated with a significantly lower risk for developing bronchiolitis and other clinically relevant adverse respiratory outcomes in the first 12 months of life in infants born to mothers with asthma. In addition, we found an inverse correlation between eNO and uEPX/c levels in infancy.

Elevated eNO levels and the number of wheezy episodes in the first year of life has been reported (RR 1.36, 95% CI 1.1 to 1.7, p=0.011)[12] but no associations were found at later time points.[12] In contrast lower eNO levels in infants who presented with respiratory symptoms within the first 6 months of life have been demonstrated (ß-coefficient −0.11, 95% CI −0.21 to−0.02, p=0.02).[26] Our results suggest that lower eNO in infancy may be of predictive value for bronchiolitis and other respiratory adverse outcomes in the first year of life.

We also found that children with higher eNO levels at 6 weeks of age were significantly less likely to present with respiratory symptoms in the first 6 weeks of life. This result is congruent with previous results reporting significantly lower eNO levels in infants with current rhinorrhoea compared with healthy infants,[17] which is opposite to what is found in adults and older children.[10] Studies have found lower eNO levels in infants with reported upper airway infections at the time of measurement,[16] and during an acute episode of wheezy bronchitis.[27] However, they were unable to comment as to whether the reduced eNO levels were pre-existing or due to the acute down-regulation of NO production during an acute viral infection. In addition, our study shows significantly lower eNO in children who develop symptoms in the first 6 weeks of life and in those who have siblings (eg, are not first born, table 1), consistent with a study showing that infants with siblings have a 20% to 30% higher chance of developing respiratory symptoms and bronchiolitis due to a greater likelihood for viral exposure.[28]

Taken together, there is mounting evidence that higher levels of eNO in infancy might be associated with a decreased risk of developing respiratory symptoms in the first year of life. eNO levels may be a surrogate for, or mechanistically related to, this reduced risk. This may involve upregulation of iNOS expression which is induced by a large number of cytokines including IL-6, TNF-alpha, IFNs, IL-4, and IL-13.[29] Therefore, higher eNO at 6 weeks of age may be an indicator of increased iNOS expression in the airways. Elevated cytokine responses may result in a lower risk for respiratory infections and symptoms in the neonatal period and in the first year of life. It is important to note that in our study eNO was not measured in those infants who had respiratory symptoms up to 2 weeks before the test. Thus, it is unlikely that the higher eNO levels in babies without respiratory symptoms in the first 6 weeks of life are the direct effect of an immune response during a respiratory tract infection. Instead, higher eNO levels may be an indicator for an elevated immune response in the absence of infection.

We found in our study a significant inverse correlation between eNO and uEPX/c. This was unexpected as eNO positively correlates with uEPX/c in school-aged children.[5 30 31] A

Table 1 Baseline subject characteristics for the complete cohort, those with valid eNO measurements, those with valid eNO measurements and questionnaire at 12 months and those with valid eNO measurements and uEPX/c available

	Complete population (n=457)	Valid eNO (n=184)	Valid eNO +Questionnaire data at 12 months (n=125)	Valid eNO +uEPX/c (n=51)
Male	53.0% (242)	57.1% (105)	59.2% (74)	72.6% (37)
Prematurity	10.1% (46)	11.4% (21)	11.2% (14)	7.8% (4)
Exclusive breast feeding	50.0% (227)	47.8% (88)	45.6% (57)	47.1% (24)
Respiratory distress at birth	7.5% (34/455)	6.0% (11/183)	6.5% (8/124)	5.9% (3/51)
Gestational age*	38.9 (38.1–40)	39.1 (38.1–39.9)	39.1 (38.1–39.9)	38.6 (37.9–39.4)
Birth weight*	3390 (3040–3720)	3365 (3000–3720)	3480 (3100–3770)	3420 (3060–3710)
Age at test (weeks)*	6 (5–7)	6 (5–7)	6 (5–7)	7 (5–7)
Weight at test*	4.8 (4.3–5.2)	4.8 (4.3–5.2)	4.9 (4.4–5.2)	5.0 (4.4–5.4)
Length at test*	56.0 (54.0–57.6)	55.5 (53.5–57.0)	55.7 (54.0–57.0)	56.0 (54.8–57.0)
Maternal asthma exacerbations in pregnancy (episodes)	20.1% (92)	19.0% (35)	23.2% (29)	27.5% (14)
First born	53.0% (242)	56.0% (103)	54.4% (68)	52.9% (27)
FeNO management group in RCT	51.9% (237)	54.9% (101)	56.8% (71)	47.1% (24)
Smoking in pregnancy (active)	10.3% (47)	10.3% (19)	8.0% (10)	11.8% (6)
Maternal exhaled carbon monoxide >6 ppm in pregnancy	18.2% (83)	13.0% (24)	8.8% (11)	13.7% (7)
Bronchiolitis (6 weeks)	6% (27/453)	6.7% (12/180)	7.3% (9/123)	9.8% (5/50)
Respiratory symptoms (before 6 weeks of age)	23.2% (99/427)	19.2% (35/182)	21.0% (26/124)	19.6% (10/51)
Bronchiolitis first 12 months	36.1% (96/266)	37.1% (46/124)	37.1% (46/124)	42.1% (16/38)
Recurrent bronchiolitis first 12 months	12.0% (32/266)	12.1% (15/124)	12.1% (15/124)	15.8% (6/38)
Wheeze first 12 months	50.6% (137/271)	48.8% (61/125)	48.8% (61/125)	57.5% (23/40)
Cold or influenza first 12 months >1	95.9% (259/270)	93.5% (116/124)	93.5% (116/124/)	94.9% (37/39)
Sneezing, blocked nose without cold or influenza first 12 months	41.9% (114/272)	42.1% (53/125)	42.1% (53/125)	30.0% (12/40)
Snored at night first 12 months	67.2% (182/271)	68.8% (86/125)	68.8% (86/125)	60.0% (24/40)
ED visit first 12 months	21.9% (59/269)	24.0% (30/125)	24.0% (30/125)	25.0% (10/40)
SABA use first 12 months	21.6% (58/269)	23.2% (29/125)	23.2% (29/125)	27.5% (11/40)

Categorical data presented as % (n); continuous data presented as median (IQR).
*Shows continous variables.
ED, emergency department; eNO, nitric oxide in exhaled air; eNO, exhaled nitric oxide; ppm, parts per million; RCT, randomised controlled trial; SABA, short-acting beta-agonists; uEPX, urinary eosinophil protein X.

dissociation between eNO and eosinophilic activation markers at 6 weeks of age could suggest that the role of type 2 immune response cytokines in the induction of iNOS is of less relevance in infancy than later in life. Of note, EPX or eosinophil-derived neurotoxin is present in all granulocytes and is not eosinophil specific, in contrast to, for instance, major basic protein. Significant quantities of EPX are present in basophils and neutrophils.[32] Higher EPX levels in serum predicted the development of recurrent wheeze after RSV-bronchiolitis.[33] We did not find an association between EPX and respiratory symptoms suggesting that eNO may be a more sensitive biomarker. The inverse correlation between eNO—that predicted lower risk for wheeze—and EPX is opposite to findings in older children.[30 34] We hypothesise that the cellular source of EPX detected in urine of infants is predominantly from neutrophilic as opposed to eosinophilic granulocytes. In support of this are findings from Malmberg *et al* who analysed inflammation in endobronchial

Table 2 Multivariable linear regression analysis* showing factors associated with eNO at 6 weeks of age

n=184	ß-coefficient (95% CI)	P value
Respiratory symptoms (before 6 weeks of age)	**−0.49 (−0.95 to −0.04)**	**0.035**
uEPX/c†	**−0.004 (−0.008 to −0.001)**	**0.021**

Bold values shows statistically signifcant results.
*R-square of the model shown was 0.10.
†Results show two independent multivariable model including uEPX/c as a variable, the model includes (n=51 subjects). eNO as outcome in this model was used as a continuous variable. Model was adjusted for male sex, prematurity, exclusive breast feeding, parity, birth weight, self-reported active maternal smoking during pregnancy and age at test in weeks.
‡ eNO, nitric oxide in exhaled air; uEPX, urinary eosinophil protein X.

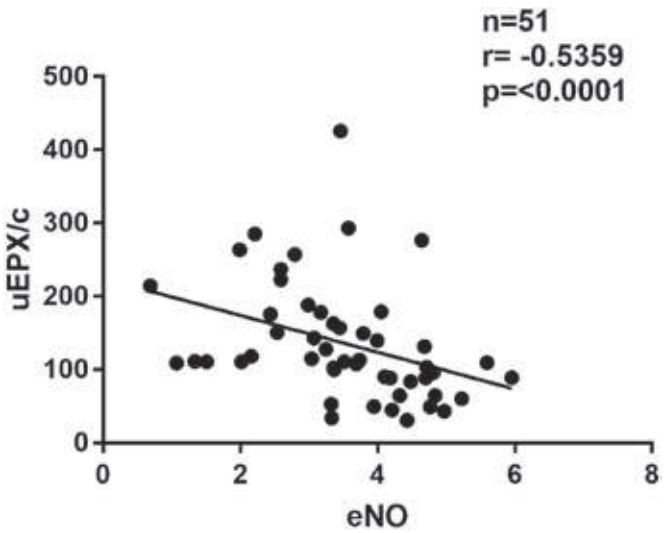

Figure 2 Correlation between urinary eosinophil protein X normalised for creatinine (uEPX/c) and exhaled nitric oxide (eNO), using Spearman rank-order correlation test (n=51).

Table 3 Multiple independent uni and multivariable models showing associations between eNO and incidence rate ratio (IRR) for symptoms before 6 weeks of age and bronchiolitis and respiratory outcomes at 12 months of age and respiratory symptoms at 6 weeks of age

	eNO		eNO adjusted	
Outcomes	**IRR (95% CI)**	**P value**	**aIRR (95% CI)**	**P value**
Bronchiolitis (n=123)	**0.82 (0.69 to 0.99)**	**0.043**	**0.82 (0.69 to 0.99)**	**0.038**
Wheeze (n=124)	**0.85 (0.73 to 0.98)**	**0.024**	**0.85 (0.73 to 0.99)**	**0.043**
Cold or influenza (n=122)	**0.96 (0.93 to 0.99)**	**0.042**	**0.94 (0.88 to 0.99)**	**0.038**
Sneezing, blocked nose without cold or influenza (n=124)	0.99 (0.83 to 1.16)	0.875	0.96 (0.80 to 1.14)	0.613
Snored at night (n=123)	0.96 (0.88 to 1.07)	0.530	0.96 (0.87 to 1.07)	0.545
Emergency department visit (n=124)	**0.80 (0.65 to 0.98)**	**0.032**	0.86 (0.70 to 1.08)	0.233
SABA use (n=123)	**0.80 (0.64 to 0.98)**	**0.036**	**0.77 (0.61 to 0.97)**	**0.028**
Recurrent bronchiolitis (n=122)	**0.77 (0.62 to 0.96)**	**0.020**	**0.76 (0.60 to 0.96)**	**0.019**
Respiratory symptoms (before 6 weeks of age)	**0.70 (0.54 to 0.94)**	**0.017**	**0.65 (0.47 to 0.90)**	**0.010**

Adjusted for infant sex, prematurity, maternal atopy, exclusive breast feeding, self-reported active maternal smoking during pregnancy, parity, mother's management group allocation in the RCT, maternal asthma exacerbation episode during pregnancy, and inhaled corticosteroid use during pregnancy.
Bold font values shows statistically significant results.
aIRR, adjusted IRR; eNO, nitric oxide in exhaled air; RCT, randomised controlled trial; SABA, short-acting beta-agonists.

biopsies and eNO in infants aged between 3 and 26 months, and showed that those with lower neutrophil counts in their airways had significantly higher eNO levels.[35]

Neutrophils are involved in first-line host defence against pathogens and are recognised to have a promiscuous role, which can range from prevention of tissue injury and preservation of homeostasis to association with RSV-induced disease severity, which may induce immune injury at the time of the infection. One of the mechanisms in which neutrophils act against pathogens[36] is through the formation of neutrophil extracellular traps (NET), activated by NF-kappa B.[36 37] NETs are able to bind to virions intercepting virus to reach target cells. NO promotes NET formation and modulates chemotaxis, adhesion, aggregation response, phagocytosis, respiratory burst and apoptosis in neutrophils.[38] Neutrophils from neonates are less capable of forming NET in response to various microbial stimuli.[39] Higher NO production in the airways of infants could, therefore, be associated with more effective responses to pathogens, including neutrophil function, which may result in an ameliorated inflammatory response and fewer symptoms.

In our study, eNO levels were measured during tidal breathing using a face mask. As shown by Franklin *et al*[13], in 88 infants studied, it is likely that breaths collected in infants from both the nose and the mouth contain mixed expired air. Therefore, eNO measured in our study is likely a mixture of nasal and airway NO, although the fractional contribution of each anatomical site to eNO is difficult to know.[13] This methodological issue may be relevant when putting our results in the context of observations made in older children and adults regarding nasal NO. Specifically, Sanders *et al* infected six non-asthmatic healthy subjects with rhinovirus and found that those subjects with higher nasal NO levels displayed less cold symptoms and more rapid virus clearance.[40] Subjects with PCD and cystic fibrosis have intrinsically low nasal NO levels of unknown cause and develop uncontrollable inflammation and failure to clear airway infections.[41] Marthin *et al* described in their study a 5% monthly increase of nasal NO until the age of 2 years in healthy infants, however, infants with PCD did not follow this increasing trend.[42] Thus, low nasal NO in infants without PCD may be a response of NO coexisting with paranasal sinus infections, due to trapped NO in obstructed paranasal sinuses or in mucus biofilms. Therefore, the association between higher eNO levels and less bronchiolitis risk in this study may be observed as a consequence of the apparent role of nasal NO in virus infection.

What has yet to be determined are the exact mechanisms that promote a switch to increased iNOS expression and eNO production in those children who develop allergic asthma. Usemann *et al* hypothesised two models of eNO metabolism during infancy.[43] The first model proposed that eNO measured at birth can be used as a biomarker for asthma risk, due to an intrinsic mechanism determined by prenatal and early postnatal risk factors only. The second model suggested that environmental factors are required to induce iNOS expression, such as respiratory infections and allergen exposure, and that eNO may only serve as a type 2 immune response biomarker after activation at some stage in early life.[43] Our data show that eNO measured at 6 weeks of age may be a marker to identify infants at higher risk for bronchiolitis and respiratory symptoms in the first year of life. eNO could be useful to identify infants at high risk and select them for research and clinical intervention in the future, however, more studies are required to investigate this potential.

Limitations of our study include that all infants were born to mothers with asthma, therefore, we cannot extrapolate our results to a general population at lower risk of respiratory symptoms in infancy. As this was a nested study where priority was given to first undertake tidal breathing and multiple breath washout measurements, we had a lower success rate of eNO compared with the other measurements. However, no selection bias was identified. In addition, some infants have already experienced an episode of bronchiolitis before eNO measurements. However, when we excluded these infants (n=11) from the analysis, the association between eNO and bronchiolitis did not change quantitatively (IRR 0.82, p=0.05). In addition, only a subset of babies had uEPX/c levels available, highlighting the difficulties in collecting urine with success being more probable in male babies. The 12-month questionnaire was not available in 33% of infants who had an eNO test, but a selection bias was not identified. Strengths are the longitudinal study design, exclusion of infants who had an acute respiratory tract infection within the 2 weeks prior to eNO testing, and online eNO measurements using chemiluminescence that could be normalised to an expiratory flow of 50 mL/s.[26]

In summary, our study shows that eNO levels were significantly higher at 6 weeks of age in infants who did not develop bronchiolitis and respiratory symptoms compared with children who did develop bronchiolitis and symptoms in the first 12 months of life. eNO levels in infancy may indicate altered immune responses in the upper and/or lower airways. Additional studies should focus on measuring eNO in infants and comprehensively determining immune responses including neutrophil activation. The role of nasal versus airway NO in infancy has yet to be further determined. eNO may be useful to identify infants at risk for adverse respiratory outcomes in the first year of life.

Author affiliations
[1]Priority Research Centre GrowUpWell, The University of Newcastle and Hunter Medical Research Institute, Newcastle, New South Wales, Australia
[2]School of Medicine and Public Health, The University of Newcastle, Callaghan, New South Wales, Australia
[3]Respiratory Medicine, The Children's Hospital at Westmead, Sydney, New South Wales, Australia
[4]Queensland Children's Medical Research Institute, University of Queensland, Herston, Queensland, Australia
[5]Priority Research Centre Healthy Lungs, The University of Newcastle and Hunter Medical Research Institute, Newcastle, New South Wales, Australia
[6]Department of Respiratory and Sleep Medicine, John Hunter Hospital, Newcastle, New South Wales, Australia
[7]Department of Paediatric Respiratory and Sleep Medicine, John Hunter Children's Hospital, Newcastle, New South Wales, Australia
Twitter Paul D Robinson @DrPLungResearch
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ORCID iDs
Carla Rebeca Da Silva Sena http://orcid.org/0000-0002-6424-7796
Vanessa E Murphy http://orcid.org/0000-0003-3282-1324
Paul D Robinson http://orcid.org/0000-0001-7397-105X
Peter D Sly http://orcid.org/0000-0001-6305-2201
Adam M Collison http://orcid.org/0000-0002-3074-3046

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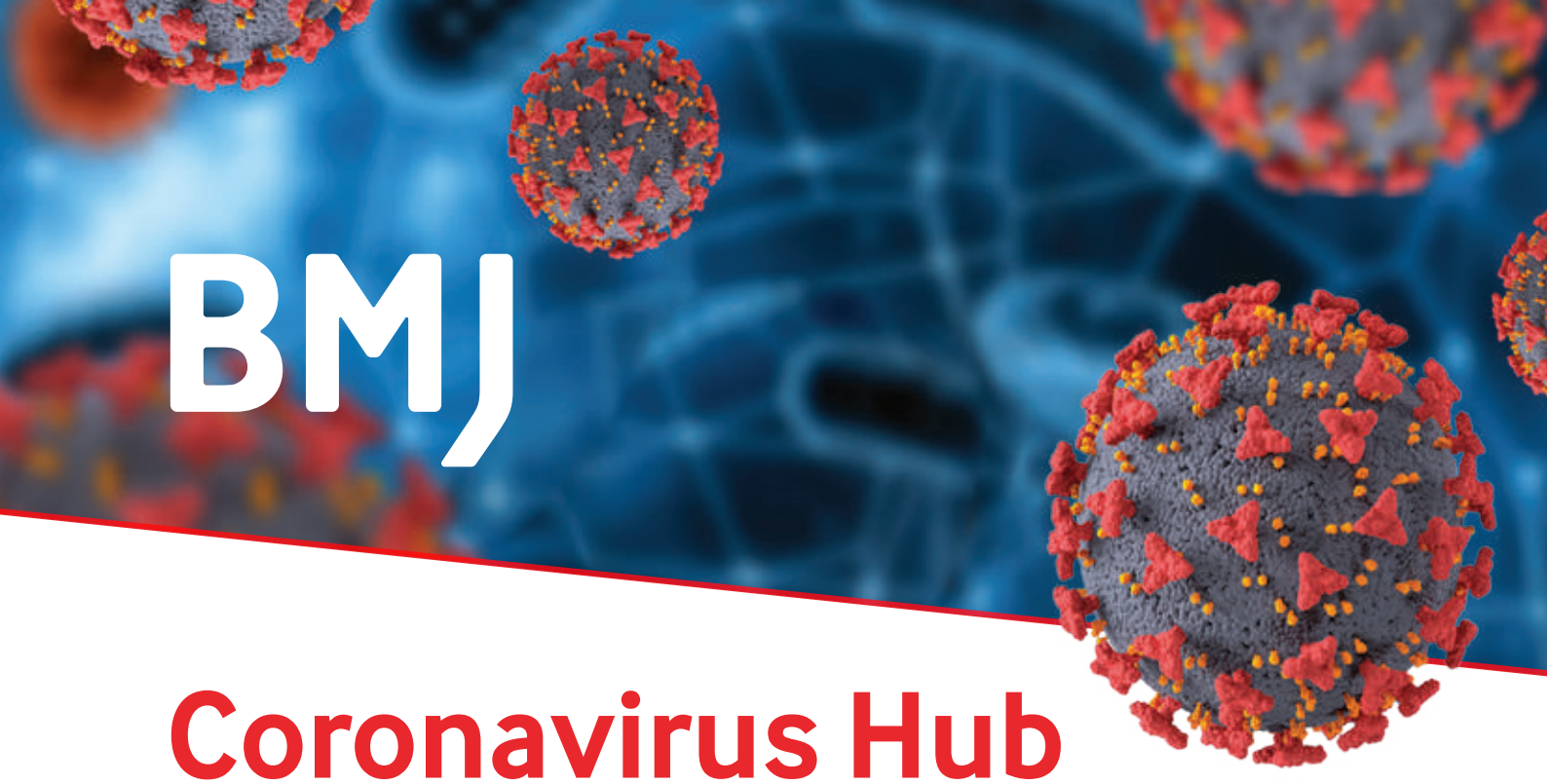

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ARTIGO ORIGINAL

Desvantagem no início de vida e asma persistente em adolescentes: um estudo de coorte no Reino Unido

Hanna Creese,[1] Eric Lai,[2,3] Kate Mason,[2] Daniela K Schlüter,[2] Sejal Saglani,[4] David Taylor-Robinson,[2] Sonia Saxena[1]

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[1]School of Public Health, Imperial College London, London, UK
[2]Institute of Population Health Sciences, University of Liverpool, Liverpool, UK
[3]Institute of Health Equity, Chinese University of Hong Kong, Hong Kong, People's Republic of China
[4]National Heart and Lung Institute, Imperial College London, London, UK

Correspondence to
Dr Hanna Creese, Imperial College London, London SW7 2AZ, UK;
h.creese@imperial.ac.uk

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RESUMO

Objetivo Determinar como os fatores de risco de início de vida explicam as desigualdades socioeconómicas na asma persistente na adolescência.

Métodos Fizemos uma análise de mediação causal recorrendo a dados de 7487 crianças e jovens do Estudo de Coorte Millennium no Reino Unido. A asma persistente foi definida como a existência de um diagnóstico relatado em quaisquer dois ou mais pontos temporais aos 7, 11 ou 14 anos. A principal exposição foi a educação materna, uma medida das circunstâncias socioeconómicas (CSE) de início de vida, utilizada para calcular o índice relativo de desigualdade. Avaliámos a forma como blocos de fatores de risco perinatais (comportamentos de saúde materna, características da criança e duração da amamentação, medidos aos 9 meses) e ambientais (condições de habitação familiar; exposição potencial a infeções através do tipo de cuidados infantis e número de irmãos e características da vizinhança, medidos aos 3 anos) mediaram o efeito total da CSE infantis no risco de asma persistente, calculando a proporção mediada e o efeito indireto natural (EIN) através de blocos de mediadores.

Resultados Aos 14 anos de idade, a prevalência global da asma persistente era de 15%. As crianças de mães com qualificações educativas mais baixas apresentavam mais probabilidade de sofrer de asma persistente, com um claro gradiente social (grau superior: 12,8% vs. sem qualificações: 20,3%). O EIN fornece o efeito das CSE atuando apenas através dos mediadores e mostra uma probabilidade aumentada de 31% de asma persistente quando as CSE são fixadas ao nível mais alto, e os mediadores ao nível que naturalmente ocorreriam nas CSE mais baixas *vs*. CSE mais elevadas (EIN OR 1,31, IC 95%, 1,04 a 1,65). Em geral, 58,9% (IC 95%, 52,9 a 63,7) do efeito total (OR 1,70, IC 95%, 1,20 a 2,40) das CSE sobre o risco de asma persistente na adolescência foi mediado por características perinatais e ambientais.

Conclusões As características perinatais e o ambiente familiar no início da vida são mais importantes na explicação das desigualdades socioeconómicas na asma persistente em adolescentes britânicos do que as exposições ambientais mais distais fora de casa.

Key messages

What is the key question?

► How does disadvantage during early-life influence inequalities in persistent asthma in adolescence?

What is the bottom line?

►Disadvantage in early-life is associated with 70% greater risk of persistent asthma in adolescents in the UK. Almost two-thirds of the excess risk is explained by perinatal and environmental mediators by the age of 3 years.

Why read on?

►This study is among the first to test the mediating role of risk factors of social inequality using robust methods for causal inference applied to a contemporary, nationally representative, birth cohort.

INTRODUCTION

Asthma is the most common chronic childhood condition in the UK and disproportionately affects health and quality of life in disadvantaged groups. School-aged children in the most deprived areas in England are two and a half times more likely to have an emergency admission for asthma than their most advantaged counterparts in 2015/2016.[1] Social gradients in children's admissions and deaths from asthma have widened in recent years.[2]

Around 8% of children and young people (CYP) aged 12–17 years are currently receiving treatment for asthma in the UK.[3] The UK has among the highest rates of asthma deaths in Europe[4] and in 20111/2012 over 25 000 CYP (aged 0–15 years) were hospitalised due to asthma.[5]

Trajectories of asthma and wheezing are hetero-geneous, and various phenotypes have been described.[6 7] For example, around 35% of preschool children with recurrent wheeze are diagnosed with asthma by 8 years.[8] The other 70% may grow out of recurrent wheeze or be diagnosed with asthma later in life. Even those diagnosed with asthma in childhood have periods of remission.[9] In this analysis, we are most interested in the pathway to persistent asthma into adolescence that is likely to track into adulthood.

Life-long lung function is influenced by environmental conditions both before and immediately after birth and in the preschool period.[10 11] Previous studies showing socioeconomic differences in wheezing during early-life point to the mediating role of early-life risk factors such as smoking during pregnancy and lower rates of breast feeding among disadvantaged groups.[12] However, the relative importance of perinatal and early years environmental characteristics as mediators of the effect of socioeconomic circumstances (SECs) in early-life on persistent asthma in adolescence in the UK remains unclear.[13]

There are several possible, modifiable, mediating pathways that link SECs in early-life to the development of persistent asthma into adolescence. Maternal smoking,[14] low birth weight,[15] premature birth,[16] not being breast fed,[17] poor housing conditions,[18] poor indoor and outdoor air quality,[19] in varying degrees, have all been found to predispose to asthma and are more common in children growing up in disadvantaged SECs. Bronfenbrenner's bioecological theory states that

the developing child is at the centre of inter-related, hierarchical systems of exposure, moving from the most proximal to the most remote.[20] To our knowledge the mediating role of these factors has not been examined in a single analysis across childhood using robust methods for causal mediation analysis, to assess their relative importance in explaining inequalities in persistent asthma in adolescence.

We therefore aimed to use causal mediation analysis to assess the relative importance of early years risk factors associated with social inequalities in persistent adolescent asthma in the UK. We hypothesised that children growing up in more disadvantaged circumstances are at increased risk of persistent asthma due to increased exposure to adverse risk factors in early life. Following Bronfenbrenner's ecological systems model,[20] we also hypothesised that proximal mediators, including perinatal and family housing conditions, would have a greater influence than exposure to infections from outside the home and neighbourhood characteristics on the risk of persistent asthma in adolescence.

METHODS

Study design and population

The Millennium Cohort Study (MCS) is a prospective cohort study of 18 818 children born in the UK between September 2000 and January 2002.[21] Participating families were randomly sampled from electoral wards, with a stratified cluster sampling design to safeguard representation of all four UK countries, disadvantaged and ethnically diverse areas. The first data collection sweep was when cohort members were approximately 9 months of age and the subsequent five sweeps of data were collected at ages 3, 5, 7, 11 and 14 years. We included all singleton children with complete data provided by the main respondent (usually the mother) who were still in the cohort at 14 years.

Outcome

We derived a longitudinal asthma variable from parental responses to the International Study of Asthma and Allergies in Childhood (ISAAC)[22] standardised question, 'Has your child ever had asthma?' asked at 7 and 11 years, and parental reports of whether their child currently has asthma at 14 years. We identified three asthma outcomes: (1) has never recorded asthma, (2) transient asthma (recorded once only at 7, 11 or 14 years) or (3) persistent (recorded at any two or more of 7, 11 or 14 years).

Exposure

Our exposure was SECs in early-life captured through highest educational qualification attained by the child's mother. This was reported at 9 months, but we assume it captures aspects of SEC during pregnancy and at birth. We first identified six groups: (1) higher degree or first-degree qualifications (reference group in regression analysis), (2) diploma in higher education, (3) A levels (exams usually taken around 18 years), (4) General Certificate of Secondary Education (GCSE, exams are usually taken around age 16 years) grades A–C, (5) GCSE grades D–G or (6) none of these qualifications.

In the second step, we calculated the relative index of inequality (RII), which compares the risk of asthma between children with the least and most disadvantaged SECs, taking into account the educational distribution, by ranking the six maternal educational groups from the lowest to the highest and allocating a score (ranging from 0 to 1) that equals the midpoint of the category's range in the cumulative distribution. The RII is a regression-based index that summarises the relative inequality across the distribution of SECs, considering the size of the population and the relative disadvantage between the different groups.[23]

Potential mediators

We conducted a review of systematic literature reviews of the social determinants of asthma across the lifecourse,[24–27] to identify four blocks of potential early-life mediators of inequalities in adolescent asthma: (1) perinatal period characteristics, including maternal health behaviours, infant characteristics and duration of breast feeding, (2) family housing conditions, (3) potential exposure to infections through childcare type and sibling number and (4) neighbourhood characteristics.

We assigned biological, social and environmental data available from the first and second data collection sweeps of the MCS (9 months and 3 years) to one of these four blocks. We ordered mediators from the most proximal, perinatal period characteristics, to the most distal, neighbourhood characteristics (box 1) in preparation for sequential modelling.

Potential confounders

We adjusted for potential confounding associated with SEC in early-life and asthma risk. These included child sex and maternal age at birth (14–24 vs ≥25 years), ethnicity (white; mixed ethnicity; Indian; Pakistani and Bangladeshi; black, or other) and atopy (including allergic predisposition and sensitisation), which was defined as maternal history of asthma or eczema diagnosis (none; either, or both). These are common antecedents of SECs, mediators and asthma.[28]

Analysis

We analysed the sample characteristics by SECs in early-life, including the proportion of CYP with persistent asthma in each group and tested univariable associations between sample characteristics and persistent asthma using multinomial regression.

Then, the analysis progressed in two stages. First, we used multinomial regression analysis to estimate the strength of association between SEC in early-life and risk for persistent asthma adjusting for blocks of potential mediators in a stepwise manner to assess how the relative risk ratio (RRR) changed on inclusion of each block of potential mediators. We adjusted all models for potential confounding by, child sex and maternal age at birth, ethnicity and atopy, as detailed in the logic model (corresponding to data available in MCS only) in figure 1. To estimate the change in RRR for mothers with the highest educational qualifications compared with those with no qualifications we calculated the difference as 100×(RRR-adjusted RRR)/(RRR-1), whereby adjusted RRRs are the different RRRs following adjustment for different mediating blocks. MCS sampling and response weights were used to account for sampling design and attrition up to the 14-year survey. The analysis was conducted using Stata V.15.

As a second step, we used counterfactual mediation analysis to quantify the inequality in persistent asthma (compared with never, transient asthma was excluded) ascribable to each block of mediators using the RII as the exposure. We estimated the ORs and 95% CIs for the natural direct effect (NDE), natural indirect effect (NIE), total effect (TE) and the proportion mediated (formulas and definitions given in online supplemental material S1) for each block of mediators, cumulatively in a stepwise manner, using the Medflex package in the R software environment.[29 30]

Sensitivity analysis

There was variation in the timing and number of times data on mediators were collected (online supplemental table S2). To capture whether the longitudinal nature of the exposure explained the proportion mediated by blocks, we conducted a sensitivity analy-sis including mediators at all the time points they were available. To examine whether environmental tobacco smoke was the main driver of home environment associations with persistent asthma, we conducted a sensitivity analysis examining environmental tobacco smoke separate from other home environment factors. To examine whether there was heterogeneity between those reporting asthma at two or three times, we conducted sensitivity analyses of the association with SEC in early-life differentiating the persistent asthma group by whether they reported asthma at two or three waves. We also did a sensitivity analysis to explore the association between SECs and current asthma at age 14 years, but not reported at previous waves.

Box 1 Description of blocks of potential mediators

1. Perinatal period measured at 9 months (first sweep, at birth)
   - Maternal pre-pregnancy body mass index (BMI): Mothers were asked at first interview, 'how tall are you (without shoes)' and 'Thinking back to just before you became pregnant with [baby], what was your weight then (without clothes)?'. Reported pre-pregnancy weight (in kilos) was then divided by height (in metres) squared to produce pre-pregnancy BMI.
   - Breastfeeding duration: Mothers were asked at first interview, 'Did you ever try to breastfeed [baby]?' and 'How old was [baby] when [s]he last had breast milk?' Answers to these questions were then used to make a duration of breastfeeding variable categorised as: (0) <3 months or (1) ≥3 months.
   - Birth weight: Birth weight of the cohort member in kilograms categorised as (0) low weight, <2.5 kg or (1) healthy weight ≥2.5 kg.
   - Gestation: Gestation time of the cohort member in days categorised as (0) not preterm, 37 weeks or more or (1) preterm, <37 weeks.
   - Maternal smoking: Mothers were asked whether they smoked any cigarettes during pregnancy: (0) non-smoker or (1) smoker.
   - Alcohol use during pregnancy: Mothers were asked about their alcohol consumption during pregnancy which is categorised as (0) never 1–2 units a month, or (1) 1 unit a week or more.
   - Wheeze: Main respondents (almost always the mother) were asked whether the cohort member had ever seen a health professional for wheeze at 9 months.
2. Housing conditions measured at 3 years
   - Damp/condensation: Respondents were asked 'How much of a problem do you have with damp or condensation on the walls in your home, apart from in the kitchen or bathroom?'. Answers were categorised as (0) no (includes 'no damp' and 'not much of a problem' and (1) yes (includes 'some problems' and 'great problem').
   - Tenure: Respondents were asked 'Do you [or your partner] own or rent your home or have some other arrangement?'. Answers were categorised as (1) own outright, (2) own—mortgage/loan, (3) part rent/part mortgage (shared equity), (4) rent from local authority, (5) rent from housing association, (6) rent privately, (7) living with parents, (8) live rent free and (9) squatting.
   - Furry pets in household: Respondents were asked 'Which of the following pets did you keep in your home during [child's] first year of life?'. Answers were grouped into (0) no pets, (1) not furry and (2) furry pets.
   - Environmental tobacco smoke: Respondents were asked 'Does anyone smoke in the same room as [child] nowadays?' Answers were coded as (0) no or (1) yes.
   - Access to garden: Respondents were asked 'do you have access to a garden?'. If they answered yes, 'Is that for your sole use or shared with anyone else?'. Answers were coded as (0) yes, sole use, (1) yes shared and (2) no.
3. Potential exposure to infection through childcare type and sibling number measured at 3 years
   - Sibling number in household: Number of siblings in the household was derived from several questions regarding the presence of biological and step siblings.
   - Informal vs formal childcare: Type of childcare was derived from questions on childcare arrangement.
4. Neighbourhood characteristics measured at 3 years

Continued

Box 1 Continued

   - Volume of traffic: Using a neighbourhood assessment form interviewers were asked 'How would you rate the volume of traffic on the street?'. Answers were coded as (1) no traffic permitted, (2) light, (3) moderate and (4) heavy.
   - Housing quality: Using a neighbourhood assessment form interviewers were asked 'How would you rate the general condition of most of the residences or other buildings in the street?'. Answers were coded as (1) well kept, good repair and exterior surfaces, (2) fair condition, (3) poor condition peeling paint broken windows and (4) badly deteriorated.
   - Rubbish on pavement: Using a neighbourhood assessment form interviewers were asked 'Is there any of the following: rubbish, litter, broken glass, drug-related items, beer cans, etc, cigarette ends or discarded packs—in the street or on the pavement?'. Answers were coded as (1) none or almost none, (2) yes some and (3) yes, just about everywhere you look.
   - Good area: Respondents were asked 'Is this a good area to bring up children?'. Answers were coded as (1) excellent, (2) good, (3) average, (4) poor and (5) very poor.

RESULTS

Subjects

There were 18 296 singleton children included in the first MCS data collection sweep at age 9 months. By age 14 years 9199 singleton children had participated in all subsequent waves. Of these children, 7487 (81%) had complete records for analysis (figure 2). See online supplemental material table S3 for the characteristics of the excluded population.

Prevalence of transient and persistent asthma by SEC

Table 1 shows that more than one in five children had one or more records of asthma at any wave (23.2%), of whom 8% had transient asthma (reported at one single time point at either 7, 11 or 14 years) and a further 15.2% had persistent asthma (recorded at any two or more of 7, 11 or 14 years). There was a clear social gradient in persistent asthma. Children born to mothers with fewer educational qualifications had a higher risk of persistent asthma by the age of 14 years (degree plus 12.8%; diploma 13.9%; A levels 14.3%; GCSE A–C 15.8%; GCSE D–G 18.9%; none 20.3%). Disadvantaged children were more likely to have a younger mother at birth, belong to an ethnic minority group and have less favourable perinatal, housing and neighbourhood characteristics.

Disadvantaged SECs in early-life was associated with a higher RRR of persistent asthma (no maternal qualifications RRR 1.73, 95% CI 1.11 to 2.69), but not transient asthma (online supplemental table S4). As there was no clear social gradient in transient asthma (online supplemental tables S4 and S5), the mediation analysis focused on the results for persistent asthma. Table 2 shows the extent to which the elevated RRR of persistent asthma by SEC in early-life was attenuated when adjusting cumulatively for blocks of mediators in a stepwise manner. Adjusting for perinatal characteristics only (Model 1) led to a 50.7% reduction in the relative risk of persistent asthma in CYP with the lowest SECs (RRR 1.35 (95% CI 0.86 to 2.12); there was no further reduction in relative risk with the addition of housing conditions (RRR 1.35, 95% CI 0.86 to 2.10, Model 2); additionally adjusting for potential infection through childcare type and sibling number (Model 3) led to a 2.8% increase of the relative risk (RRR 1.37 (95% CI 0.87 to 2.16)), and additional adjustment for neighbourhood conditions (Model 4) led to a further 8.5% reduction in relative risk (RRR 1.31 (95% CI 0.82 to 2.09)). In this final model, adjusting for all blocks together, the overall reduction in RRR was 56.3%.

We repeated the regression analysis including mediators available at all time points (9 months, 3 and 5 years, (online

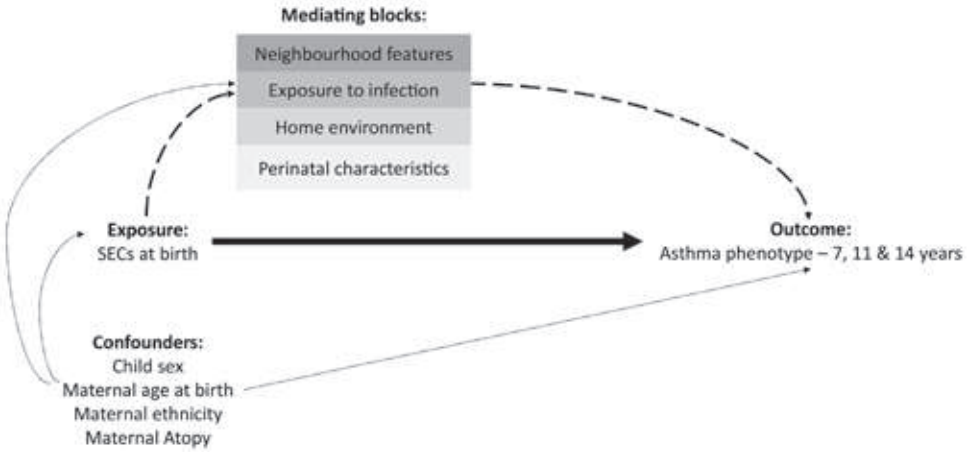

Figure 1 Logic model of the pathways from SECs at birth (maternal education captured at 9 months) to mid-childhood/adolescent asthma (7, 11 and 14 years), with direct pathway shown in bold, indirect pathways via mediators in dashed lines and baseline confounding pathways in dotted lines. Mediating blocks capture: (1) Perinatal characteristics at 9 months (maternal pre-pregnancy body mass index; breastfeeding duration; birth weight; gestation; maternal smoking; alcohol use during pregnancy; seen a health professional for wheeze?), (2) home environment at 3 years (damp/condensation; tenure; furry pets in household; environmental tobacco smoke; access to garden) (3) exposure to infection at 3 years (sibling number in household and informal vs formal childcare) (4) neighbourhood characteristics at 3 years (volume of traffic; housing quality; rubbish on pavement; good area to bring up children?). This logic model corresponds to data available in Millennium Cohort Study only and is not a complete directed acyclic graph. SECs, socioeconomic circumstances.

supplemental table S2) in supplementary material details the availability of potential mediators by wave). Results were similar to that of the main analysis (online supplemental table S6). An additional analysis examining environmental tobacco smoke (ETS) separate from other home environment factors was conducted. After taking into account the attenuation of risk after adjusting for the perinatal block, there was no additional attenuation after adjusting for ETS exposure (online supplemental table S7). We repeated the regression analysis differentiating the persistent asthma group by whether they reported asthma at two or three waves. Results were also similar to that of the main analysis (online supplemental table S8). Sensitivity analysis to explore the association between SECs and current asthma at age 14 years found no association (online supplemental table S9).

Counterfactual mediation analysis results

For the second step of the analysis, the counterfactual mediation analysis results are shown in tables 3 and 4 and figure 3. The NDE (OR 1.27, 95% CI 0.62 to 2.63) is the increase in the likelihood of persistent asthma, comparing low-to-high SECs that we would observe if the levels of mediators remained as those for the children in the most advantaged SECs (formulas given in online supplemental material S1). The NIE is the increased likelihood of persistent asthma we would see in the low SEC group if the mediators take on the values they would naturally have if the children had been in the high SEC group (OR 1.31, 95% CI 1.04 to 1.65). The TE, the sum of the NDE and the NIE even in presence of exposure-mediator interaction, had an OR of 1.70 (95% CI 1.20 to 2.40).

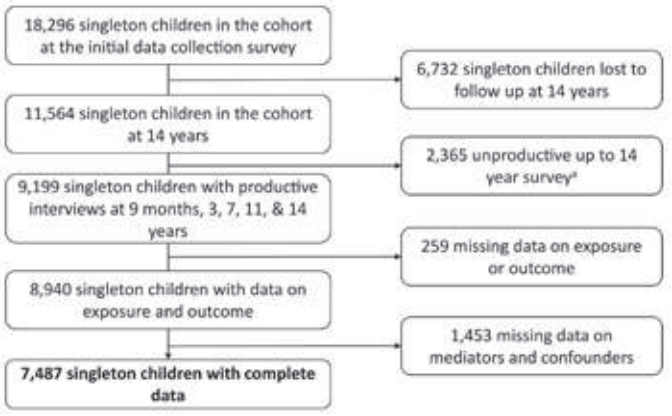

Figure 2 Flow diagram of cohort construction a productive cohort members have some data from one of six collection questionnaires at every data collection wave.

Findings from the counterfactual mediation analysis show the proportion mediated by each block for persistent asthma compared with never by RII (table 4): Perinatal characteristics alone mediated 37.5% (95% CI 30.6 to 43.3); with the addition of housing conditions 61.2% was mediated (95% CI 56.0 to 65.4); additionally adjusting for potential infection through childcare type and sibling number mediated 56.1% (95% CI 50.2 to 60.9), and after additional adjustment for neighbourhood conditions we found that 58.9% (95% CI 52.9 to 63.7) of the TE of SECs in early-life on the likelihood of persistent asthma in CYP was mediated through collective exposure to all mediating blocks (see table 4).

DISCUSSION

Main findings

The prevalence of persistent asthma in the most disadvantaged British adolescents was 20%, compared with 13% for the most advantaged. Being born into disadvantaged SECs increased the likelihood of developing persistent asthma by 70%. Almost two-thirds (58.9%) of the excess risk attributable to perinatal and environmental exposures by the age of 3 years. Consistent with the Bronfenbrenner model, we found that the proximal environment was somewhat more impactful on persistent asthma risk than the external environment beyond the household, such as potential exposure to infection through childcare type and sibling number and neighbourhood characteristics. In contrast, we did not see a social gradient in transient asthma, which affected 8% of adolescents.

Comparison with other studies

Our findings of a social gradient in persistent asthma are supported by previous studies that reported inequalities in children who have symptoms of severe asthma[31–33] and those which lead to hospital admissions.[2] For example, Case *et al* found that disadvantaged children with asthma in the USA were more likely to have symptoms of severe asthma than advantaged counterparts, with the inequality greater for older children.[32] Yet, our study is one of the first to assess how early-life risk factors explain the inequality in persistent asthma in adolescence using counterfactual mediation analysis applied to data from birth to adolescence. We have added support to the notion that the perinatal period and initial years of life are a critical period to influence lifelong inequalities in lung development[10 11] and explained in part why the asthma epidemic in the UK disproportionately impacts children in disadvantaged SECs.

Previous studies have shown that less favourable perinatal and environmental exposures in early-life, predict later respiratory health.[12 14] Our findings add weight to the importance of maternal health even before birth and subsequent perinatal characteristics

Table 1 Characteristics (%) of the study population by maternal educational qualification at birth (N=7487)

%	Degree plus	Diploma	A levels	GCSE A–C	GCSE D–G	None	Total
Asthma							
None	79.0	78.8	77.7	75.7	70.7	72.7	76.8
Transient	8.2	7.3	8.0	8.5	10.4	6.9	8.0
Persistent	12.8	13.9	14.3	15.8	18.9	20.3	15.2
Child's sex							
Boys	48.5	50.7	48.5	46.8	50.7	50.4	49.3
Girls	51.5	49.3	51.5	53.2	49.3	49.6	50.7
Maternal age at birth							
≥25 years	97.8	93.1	74.6	75.7	60.5	63.6	84.3
14–24 years	2.2	6.9	25.4	24.3	39.5	36.4	15.7
Maternal ethnicity							
White	89.0	93.4	93.5	94.0	92.2	71.9	92.2
Mixed	0.9	0.6	0.5	0.4	0.6	1.1	0.6
Indian	4.1	1.8	1.0	1.0	1.1	2.7	1.8
Pakistani	2.0	1.2	2.9	2.8	5.4	16.6	2.7
Black	2.2	1.9	1.3	1.3	0.8	4.5	1.7
Other	1.7	1.1	0.4	0.4	0.1	3.2	1.0
Maternal atopy							
None	75.1	69.9	69.6	69.7	67.8	73.2	70.3
Eczema or asthma	19.8	24.5	23.0	24.1	24.2	20.9	23.5
Both	4.3	5.9	7.5	7.0	11.4	5.8	6.7
Maternal pre-pregnancy body mass index							
Healthy weight	71.0	61.5	53.9	52.8	51.0	46.2	58.2
Underweight	2.8	2.7	3.8	3.9	5.6	7.6	3.5
Overweight	18.8	24.8	27.4	27.4	23.8	29.9	25.4
Obese	7.4	11.1	14.9	15.9	19.5	16.3	13.0
Breast fed for at least 3 months							
No	59.5	62.4	74.3	78.3	91.4	87.2	69.7
Yes	40.5	37.6	25.7	21.7	8.6	12.8	30.3
Child's birth weight							
Low weight	2.8	4.8	5.7	6.5	5.4	1.2	5.5
Healthy weight	97.2	95.2	94.3	93.5	94.6	88.2	94.5
Gestational age at birth							
Not preterm	95.2	93.8	93.5	92.6	94.2	90.0	93.5
Preterm	4.8	6.2	6.5	7.4	5.8	10.1	6.5
Maternal smoking in pregnancy							
No	88.0	82.3	67.3	55.5	48.0	46.6	71.7
Yes	12.0	17.7	32.7	44.5	52.0	53.4	28.3
Alcohol consumption during pregnancy							
≤1 unit per week	85.4	86.1	90.9	92.1	93.1	92.7	88.7
Any unit per week	14.6	13.9	9.1	7.9	6.9	7.3	11.3
Wheeze at 9 months							
No	97.6	96.8	96.9	96.5	96.0	93.3	96.7
Yes	2.4	3.2	3.1	3.5	4.0	6.7	3.3
Damp in the house							
No	89.8	89.4	87.5	86.6	79.7	75.8	87.6
Yes	10.2	10.6	12.5	13.4	20.3	24.3	12.5
Tenure							
Own outright	8.1	5.1	4.3	3.7	5.2	6.6	5.0
Own mortgage	87.1	84.2	69.2	59.8	34.1	21.2	72.1
Part rent/mortgage	0.2	0.4	1.5	0.6	0.6	0.1	5.9

Continued

Table 1 Continued

%	Degree plus	Diploma	A levels	GCSE A–C	GCSE D–G	None	Total
Local authority	0.5	2.5	10.4	15.7	32.3	40.1	9.2
Housing association	0.1	2.2	6.0	11.0	15.0	19.2	5.7
Privately rented	2.3	4.1	6.0	7.0	10.8	10.9	5.4
Living with parents	1.0	1.0	2.1	1.9	1.4	1.3	1.4
Rent free	0.8	0.6	0.5	0.3	0.6	0.7	0.5
Furry pets							
None	59.7	52.2	46.8	42.5	45.5	54.3	49.7
Not furry	7.1	9.3	9.3	10.2	7.4	11.9	9.4
Yes	33.2	38.4	43.9	47.2	47.1	33.8	40.9
Environmental tobacco smoke							
No	97.6	93.3	84.8	79.3	67.8	62.8	87.0
Yes	2.4	6.7	15.2	20.7	32.3	37.2	13.0
Garden access							
Yes, sole access	94.3	93.7	89.0	87.1	82.3	78.3	90.4
Yes, shared	2.6	2.2	3.0	2.4	4.3	4.0	2.6
No	3.1	4.2	8.0	10.5	13.4	17.7	7.0
Sibling number							
0	18.2	24.0	29.3	24.8	22.7	18.0	24.2
1	58.7	53.9	49.1	45.4	41.3	35.0	50.4
2 or more	23.1	22.2	21.6	29.8	36.1	47.0	25.4
Childcare							
Informal	62.0	76.7	88.6	91.8	96.7	99.5	82.3
Formal	38.0	23.3	11.4	8.2	3.3	0.5	17.7
Condition of buildings							
Good condition	78.6	75.2	59.5	52.1	35.0	22.3	64.2
Fair condition	20.9	23.4	37.6	42.4	56.0	63.5	32.5
Poor condition	0.5	1.3	2.9	5.1	8.8	13.4	3.1
Bad condition	0.1	0.1	0.1	0.5	0.2	0.8	0.2
Volume of traffic							
None	3.7	4.7	5.8	4.3	5.8	5.5	4.8
Light	75.6	76.1	75.2	77.2	70.8	72.2	75.8
Moderate	15.8	14.3	14.4	13.9	17.0	16.1	14.5
Heavy	4.9	4.9	4.6	4.6	6.3	6.2	4.9
Litter in the street							
None	92.7	90.2	81.4	73.2	58.4	45.3	82.2
Some	7.2	9.3	17.7	24.7	38.2	47.4	16.5
Everywhere	0.1	0.6	0.9	2.1	3.4	7.3	1.3
Good area							
Excellent	50.0	44.0	31.6	27.9	17.1	16.0	36.8
Good	38.0	40.8	40.7	39.5	40.1	35.6	40.0
Average	9.9	12.8	20.5	24.0	29.1	31.5	17.6
Poor	2.1	1.9	5.1	5.6	8.0	10.1	3.8
Very poor	0.1	0.6	2.2	3.0	5.7	6.8	1.8

GCSE, General Certificate of Secondary Education .

on later health, including maternal pre-pregnancy body mass index, smoking during pregnancy, preterm birth, birth weight, breast feeding and early wheeze, which accounted for 37.5% of the association between SEC in early-life and persistent asthma by adolescence. Previous studies have shown these characteristics are independent predictors of lung development[12 14–16] and in this study, we have formally tested their mediating role in explaining inequalities in persistent asthma. We found that the quality of the home environment is important in the development of inequalities in persistent asthma in adolescence, accounting for a further 23.7%, which supports previous study findings of associations between dampness or ETS and children's asthma.[18]

In contrast to studies that have found associations between childcare type,[34] sibling number[35] and neighbourhood characteristics[36] and persistent asthma, additional adjustment of these characteristics did not further mediate the association between SEC in early-life and persistent asthma. This is not to say that more distal environmental conditions are not important in the development of inequalities in persistent asthma, rather that once conditioned on perinatal period characteristics and the home environment there

Table 2 Relative risk ratios (RRR) for transient and persistent asthma compared with never by SEC adjusted for blocks of mediators (N=7487)

	Baseline model		Model 1		Model 2		Model 3		Model 4	
	RRR*	95% CI	RRR	95% CI	RRR	95% CI	RRR	95% CI	RRR	95% CI
Transient asthma										
Diploma	0.86	0.62 to 1.19	0.81	0.58 to 1.12	0.81	0.58 to 1.12	0.79	0.56 to 1.10	0.78	0.55 to 1.09
A levels	0.93	0.65 to 1.34	0.83	0.57 to 1.21	0.82	0.57 to 1.19	0.79	0.54 to 1.15	0.76	0.52 to 1.12
GCSE A–C	1.01	0.69 to 1.47	0.87	0.59 to 1.27	0.85	0.57 to 1.26	0.81	0.55 to 1.20	0.77	0.52 to 1.15
GCSE D–G	1.26	0.74 to 2.14	1.09	0.65 to 1.85	1.08	0.64 to 1.83	1.03	0.61 to 1.74	0.96	0.57 to 1.63
None	0.92	0.56 to 1.51	0.73	0.44 to 1.23	0.71	0.42 to 1.21	0.68	0.40 to 1.15	0.63	0.38 to 1.05
Persistent asthma										
Diploma	1.03	0.78 to 1.36	0.96	0.72 to 1.27	0.96	0.73 to 1.27	0.95	0.72 to 1.26	0.95	0.72 to 1.26
A levels	1.08	0.78 to 1.50	0.96	0.69 to 1.35	0.97	0.69 to 1.36	0.96	0.68 to 1.35	0.95	0.67 to 1.33
GCSE A–C	1.24	0.91 to 1.70	1.09	0.79 to 1.51	1.10	0.80 to 1.52	1.10	0.79 to 1.53	1.08	0.78 to 1.49
GCSE D–G	1.50	0.95 to 2.35	1.34	0.85 to 2.12	1.35	0.86 to 2.13	1.36	0.85 to 2.15	1.31	0.83 to 2.07
None	1.71	1.07 to 2.74	1.35	0.86 to 2.12	1.35	0.86 to 2.10	1.37	0.87 to 2.16	1.31	0.82 to 2.09
Proportion attenuated (%)†										
			50.70		50.70		47.89		56.34	

Model 1: Baseline model +perinatal characteristics (maternal pre-pregnancy body mass index; breastfeeding duration; birth weight; gestation; maternal smoking during pregnancy; alcohol use during pregnancy; wheeze.
Model 2: Model 1+housing conditions (damp; tenure; furry pets; environmental tobacco smoke; access to garden).
Model 3: Model 2+potential exposure to infection (sibling number; childcare type).
Model 4: Model 3+neighbourhood characteristics (volume of traffic; housing quality; rubbish; area rating).
*All models were adjusted for baseline confounders (child sex, maternal age at birth, maternal ethnicity and maternal atopy). Degree plus is the reference category.
†Proportion of RRR of persistent asthma attenuated by comparison of baseline model with models 1–4 for lowest SECs (100×(RRR-adjusted RRR)/(RRR-1))
GCSE, General Certificate of Secondary Education ; SEC, socioeconomic circumstances .

was no additional mediation. This could be because more distal environmental conditions are downstream ancestors in the causal pathway from perinatal characteristics and the home environment.

Strengths and limitations

The significant strengths of our study include the use of data from a large, representative UK cohort, longitudinal follow-up from birth to adolescence and the inclusion of a range of socially patterned risk factors for asthma operating in the early years. A key strength of our analysis is the application of a modern approach to mediation analysis applied to high resolution cohort data to better understand mediating pathways to address health inequalities in asthma. These methods allow us to unpick the mediating pathways linking adverse SECs to outcomes and identify those which appear to be most important. The approach used addresses the limitations of traditional multivariable regression methods for assessing mediation which assumes a linear relationship and no interaction between the exposure and mediator, and, that there is no intermediate confounding. This study is among the first to formally test the mediating role of risk factors of social inequality using counterfactual methods.

However, as with all observational studies, there are several important sources of potential bias to consider. The MCS at baseline had a representative sample of 18 818 children born in the UK between September 2000 and January 2002. Yet, by age 14 years 9199 singleton children had participated in all subsequent waves. To minimise attrition bias, we used response weights to account for the loss of respondents up to age 14 years.[37] The attrition weights adjust the sample composition to take account of the selective loss of respondents, for example, low income families who may be less likely to remain in the cohort. More information on the MCS attrition weights can be found on the Centre for Longitudinal Studies website (https://cls.ucl.ac.uk/cls-studies/millennium-cohort-study/). Of the children still in the study at 14 years, 81% had complete data and were included in our analysis. Nevertheless, children with missing data were more likely to be from disadvantaged SECs which may underestimate associations between SECs in early-life and asthma. The MCS oversampled from minority ethnic groups, but our focus was not to understand ethnic health inequalities. In our study sample maternal ethnicity was predominantly white hence we caution against generalising our findings across all ethnic groups and recommend more research to understand ethnic health inequalities in persistent asthma.

Although we used advanced methods for causal mediation analysis and adjusted for a range of potential confounders, the assumption of complete adjustment of confounding is still required for a causal interpretation of our estimates. The total causal effect of SEC in early- life on asthma needs to be interpreted with caution due to likely residual confounding by historic parental SECs. While we partially take genetic predisposition into account in our analysis by adjusting for maternal history of asthma or eczema and

Table 3 Relative risk ratios (RRR) for persistent asthma by relative inequality index adjusted for blocks of mediators (N=7487)

	Baseline model		Model 1		Model 2		Model 3		Model 4	
	RRR*	95% CI	RRR	95% CI	RRR	95% CI	RRR	95% CI	RRR	95% CI
Transient asthma										
RII	1.25	0.83 to 1.90	1.03	0.67 to 1.58	1.00	0.64 to 1.55	0.96	0.62 to 1.48	0.89	0.58 to 1.36
Persistent asthma										
RII	1.70	1.21 to 2.40	1.45	1.03 to 2.06	1.45	1.02 to 2.05	1.49	1.04 to 2.14	1.41	0.98 to 2.03

Model 1: Baseline model +perinatal characteristics (maternal pre-pregnancy body mass index; breastfeeding duration; birth weight; gestation; maternal smoking during pregnancy; alcohol use during pregnancy; wheeze.
Model 2: Model 1+housing conditions (damp; tenure; furry pets; environmental tobacco smoke; access to garden).
Model 3: Model 2+potential exposure to infection (sibling number; childcare type).
Model 4: Model 3+neighbourhood characteristics (volume of traffic; housing quality; rubbish; area rating).
*All models were adjusted for baseline confounders (child sex, maternal age at birth, maternal ethnicity and maternal atopy).
RII, relative index of inequality .

Table 4 NDE, NIE, TE and proportion mediated for persistent asthma compared with never by socioeconomic circumstance (N=7487)

Blocks of mediators	Effects	OR	95% CI	Proportion mediated %	95% CI
Perinatal characteristics	NDE	1.43	0.72 to 2.84	37.5	30.6 to 43.3
	NIE	1.18	0.98 to 1.42		
	TE	1.70	1.20 to 2.40		
+Home environment at 3 years	NDE	1.26	0.63 to 2.50	61.2	56.0 to 65.4
	NIE	1.32	1.03 to 1.70		
	TE	1.70	1.20 to 2.40		
+Exposure to infection at 3 years	NDE	1.29	0.64 to 2.63	56.1	50.2 to 60.9
	NIE	1.29	1.00 to 1.66		
	TE	1.70	1.20 to 2.40		
+Neighbourhood characteristics at 3 years	NDE	1.27	0.62 to 2.63	58.9	52.9 to 63.7
	NIE	1.31	1.04 to 1.65		
	TE	1.70	1.20 to 2.40		

NDE, natural direct effect; NIE, natural indirect effect; TE, total effect.

ethnicity, we were unable to adjust fully for genetic risk factors for asthma.

Our exposure of SECs in early-life is reported at 9 months, but we assume maternal educational level to be representative of SECs during pregnancy and at birth. We do not expect maternal educational level to have changed much during this period, which is why we can justify looking at mediators during pregnancy. Maternal educational level is linked to general and health-related knowledge, literacy, prestige, earning capacity and self-efficacy. It has been used to measure family SECs in social epidemiological studies,[12 38 39] is a more stable measure of SECs than income which may fluctuate over time, and has a greater impact on child health than paternal education.[40]

The limited data on asthma persistence is a potential area of bias that may underestimate the association between SECs in early-life and persistent asthma into adolescence. We derived a longitudinal asthma variable from parental responses to the standardised ISAAC[22] question, 'Has your child ever had asthma?' asked at 7 and 11 years, and parental reports of whether their child currently has asthma at 14 years. There was variation between those reporting ever asthma at 7 or 11 years, with almost a third (29%) of those reporting ever asthma at 11 previously reporting no to ever asthma at 7 years. Current asthma at 14 with reported asthma at either 7 or 11 alone did not capture all variations in reporting of persistent asthma between 7 and 14 years, as 10% of those reporting no asthma at 14 years had reported having asthma at 7 or 11 years. Therefore, persistent asthma was defined as any two or more reports of asthma at 7, 11 or 14 years.

As asthma diagnoses are unreliable in early childhood,[41] we can be more confident in our outcome of reported asthma diagnosis from 7 years onwards. The prevalence of persistent asthma in our analysis (15%) was higher than that recorded in UK health records for adolescents (8%).[3] However, this is in line with previous study findings that parent-reported asthma prevalence is greater than general practitioner (GP) recorded asthma diagnosis.[42] Griffiths and colleagues suggest several reasons for why this may arise: parents may not take their child to the GP for asthma symptoms, the GP may record a different diagnosis, or may not record any diagnosis, or parents may report other breathing sounds as asthma. Parent-reported asthma is commonly used in epidemiological studies[12 25 31 34 43] and analysis has shown good agreement between parental and offspring asthma reports, making it a useful tool in the absence of GP recorded asthma or objective measurement such as spirometry or peak flow.[44]

Understanding complex causal pathways is challenging. Mediating blocks are expected to cluster and are difficult to interpret in isolation. We, therefore, took a sequential 'en-block' mediation approach and analysed the cumulative effect of blocks, considering

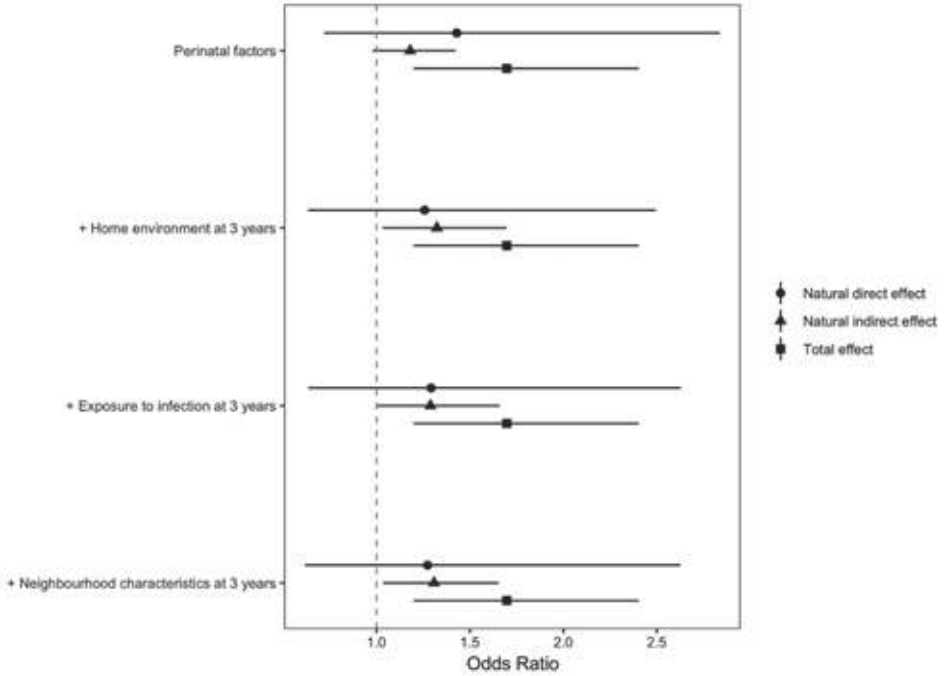

Figure 3 Mediation analysis with a counterfactual approach by cumulative blocks of mediators (perinatal characteristics, housing conditions, potential exposure to infection and neighbourhood) characteristics in the association between socioeconomic circumstance and persistent asthma.

proximal to distal mediators.[29] Perinatal characteristics were the most influential block of mediators in our analysis, and we can be relatively confident about the proportion mediated through this most proximal block as a whole, while not able to isolate the relative contribution and the temporal sequence of each individual perinatal measure in this block. Furthermore, we can also be confident about the total proportion mediated by all the blocks combined, while the proportion attributable to each block individually is challenging to assess due to the interaction between these pathways.

Some of the measures in mediating blocks are subject to change over early-life. Our data gathered at the age of 3 years provides a snapshot of housing, potential exposure to infection and neighbourhood characteristics. These conditions may change throughout early-life, and potentially more so for disadvantaged families with housing insecurity.[45] It is also important to note that the assessment of neighbourhood characteristics is subjective as it includes interviewer observations of volume of traffic, housing quality, littering and area quality on the day of the interview.

The mediating block of potential exposure to infections through childcare type and sibling number does not capture children's history of infection, rather it is a proxy measure of exposure to children outside the home. Respiratory infections which cause wheeze in early-life are associated with later asthma.[46] To partially account for this, we include parental reports of whether children have seen a health professional for wheeze at 9 months in the perinatal mediating block.

Policy implications

Our results support the need for an early prevention strategy to focus on ensuring optimal conditions during pregnancy and childhood to support the healthy development of children and reduce inequalities across the lifecourse. This is currently advocated in Public Health England's strategy for giving children the best start in life,[47] yet, much of the strategy is focused on individual actions and provision of health services for mothers and CYP rather than structural and social determinants of respiratory health.

Through improved understanding of early-life pathways, GPs can better identify children in need of closer monitoring throughout childhood and support families to reduce the risk of persistent asthma into adolescence. However, while quality healthcare is crucial for CYP with asthma, our findings demonstrate the importance of acting to reduce child poverty.

Interventions need to mitigate the effects of disadvantageous SEC in early-life by acting on perinatal and home environment mediating pathways. For the perinatal pathway, this should be through support for smoking cessation in pregnancy and robust systems to support breast feeding delivered through midwifery, children's centres and ensuring all hospitals are accredited with UNICEF's baby friendly initiative.[48] For the home environment pathway, this should be through improving housing conditions in disadvantaged areas and education on the impacts of damp and ETS on respiratory health.

In conclusion, we found that perinatal characteristics and the home environment in early life are more important in explaining socioeconomic inequalities in persistent asthma in British adolescents than more distal environmental exposures outside the home.

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ORCID iDs
Hanna Creese http://orcid.org/0000-0003-0431-7753
Eric Lai http://orcid.org/0000-0002-1229-9471
Kate Mason http://orcid.org/0000-0001-5020-5256
Daniela K Schlüter http://orcid.org/0000-0001-9018-0022
Sejal Saglani http://orcid.org/0000-0001-5192-6418
David Taylor-Robinson http://orcid.org/0000-0002-5828-7724
Sonia Saxena http://orcid.org/0000-0003-3787-2083

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ARTIGO ORIGINAL

Análise custo-benefício da azitromicina para a prevenção de agudizações agudas de doença pulmonar obstrutiva crónica

Safa Ahmadian,[1] Don D Sin,[2,3] Larry Lynd,[1] Mark Harrison,[1] Mohsen Sadatsafavi[1,2]

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[1]Respiratory Evaluation Sciences Program, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia, Canada
[2]Centre for Heart Lung Innovation, The University of British Columbia, Vancouver, British Columbia, Canada
[3]Faculty of Medicine, The University of British Columbia, Vancouver, British Columbia, Canada

Correspondence to
Dr Mohsen Sadatsafavi, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada; msafavi@mail.ubc.ca

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RESUMO

Contexto A terapia com azitromicina oral de dose reduzida está recomendada como tratamento preventivo para agudizações agudas da DPOC. No entanto, o balanço global do custo-benefício deste tratamento ainda não foi bem estudado.

Métodos Foi criado um modelo Markov probabilístico de DPOC para simular o curso da DPOC ao longo de 20 anos. O modelo foi povoado com evidências da literatura e uma análise de dados dedicada. O benefício da azitromicina foi modelado como uma redução nas taxas de agudização. Foram considerados eventos adversos, incluindo eventos cardiovasculares, perda de audição, sintomas gastrointestinais e resistência antimicrobiana (conduzindo a um declínio gradual da eficácia da azitromicina). A todos os resultados foi atribuído um peso útil relacionado com a saúde para estimar a mudança líquida global em anos de vida ajustados pela qualidade (QALY) associados ao uso da azitromicina.

Resultados Em pacientes com um histórico de agudização positiva, a azitromicina resultou num ganho líquido de QALY de 17,9 por 100 pacientes (99,8% de probabilidade de ganho de QALY esperado) ao longo de 20 anos. O benefício líquido aumentou para 21,8 QALYs por 100 doentes (99,9% de probabilidade de ganho de QALY esperado) no subgrupo "exacerbador frequente". A azitromicina não apresentou benefícios líquidos entre aqueles sem qualquer agudização moderada/grave no ano anterior. Os achados mostraram-se robustos contra séries de análises de sensibilidade, de cenários e de limiares.

Conclusões A terapia a longo prazo com azitromicina confere um benefício líquido a pacientes ex-fumadores com DPOC com um histórico recente de agudizações e um benefício ainda maior para as agudizações frequentes.

Key messages

What is the key question?

►What is the benefit–harm balance of maintenance azithromycin therapy in patients with COPD, and which subgroups benefit the most from it?

What is the bottom line?

► Daily maintenance azithromycin therapy is very likely net-beneficial over 20 years among patients with COPD who are former smokers and have a recent history of exacerbations, especially those who tend to exacerbate frequently.

Why read on?

►Contemporary guidelines express concerns about adverse effects of long-term maintenance azithromycin therapy; our analysis shows that guideline-recommended azithromycin therapy is very likely to be net beneficial, a finding that remained robust to several assumptions regarding the long-term effectiveness and adverse event risks of this treatment.

INTRODUCTION

COPD is a common inflammatory lung disorder that is characterised by persistent airflow limitation and periods of acute worsening of respiratory symptoms, called exacerbations.[1] With 3.23 million deaths reported in 2019 alone,[2] COPD is a leading cause of morbidity and mortality around the world.[1] COPD exacerbations are a major cause of medical hospital admissions across many jurisdictions.[3]

Prevention of exacerbations is a major goal in COPD management. Pharmacotherapy is a central component of such prevention. A large randomised controlled trial (RCT) involving 1142 subjects has shown that daily use of low-dose azithromycin therapy, a broad-spectrum antibiotic with immunomodulatory properties, reduces the rate of exacerbations by 27%.[4] Based on such results, the use of maintenance azithromycin is currently recommended for patients who continue to exacerbate despite being on maximal inhaled therapies. However, daily use of azithromycin is associated with side effects, including antibiotic resistance,[5] impaired hearing,[4] cardiovascular (CV) events[6] and gastrointestinal (GI) symptoms,[5] and there have been concerns about whether the risk of such adverse events outweighs the benefit of azithromycin over the long term.[5] In light of such concerns, an objective evaluation of the benefits and harms of azithromycin is warranted.

To the best of our knowledge, the benefit–harm balance of azithromycin has not been objectively studied in COPD. A benefit–harm analysis is a method for quantitative assessment of the overall value of a treatment based on consideration of its benefits and side-effects. This framework provides an objective and transparent mechanism for combining the various outcomes of treatment into a single 'net benefit' metric that can inform clinical decision-making.[7] The primary objective of this study was to perform a probabilistic benefit–harm analysis of long-term, low-dose daily azithromycin for the prevention of exacerbations in patients with moderate to severe COPD. Since COPD is a heterogeneous disease, the net benefit of azithromycin can vary according to patient characteristics (specifically based on their exacerbation history). Therefore, our secondary objective was to identify patient subgroups that are most likely to receive a net benefit from azithromycin.

METHODS

We conducted a probabilistic model-based benefit–harm analysis of prophylactic azithromycin (250

mg/d) to prevent COPD exacerbations among patients who are already on maximum inhaled therapy. The benefit of azithromycin (exacerbation reduction) and its harm (adverse events) were transformed into quality-of-life weights to generate a single metric of net changes in quality-adjusted life years (QALYs).

The baseline time horizon of the study was 20 years and outcomes were discounted at 3% per year (both parameters were subjected to sensitivity analysis).[8] The overall characteristics of the population were adapted from the MACRO study, the largest and most conclusive RCT of prophylactic azithromycin in COPD.[4] In line with this study, patients with moderate to severe COPD (defined as Global Initiative for Chronic Obstructive Lung Disease (GOLD) severity grades II–IV) with at least one moderate or severe exacerbation in the previous year were considered. Following MACRO's inclusion criteria, we also assumed that patients with corrected QT (QTc) interval prolongation are excluded from treatment, and so are patients with existing hearing impairment or concomitant asthma. Because the majority of MACRO patients were from the USA, for consistency, we prioritised US-based studies for evidence synthesis.

Model

We created a probabilistic discrete-time Markov model to simulate the natural history of COPD and the effectiveness of azithromycin and its side effects. The core states of the model were based on GOLD severity grades II–IV. These states were chosen because there are robust data on the rate of transition across GOLD grades over time and on their relationship with exacerbations.[9 10] We further subdivided each state into 'no long-term adverse effects' and 'long-term adverse effects' to model the possible long-lasting side effects of azithromycin. The cycle length of the model was 1 year. A schematic illustration of the model is provided in figure 1. Input parameters are provided in table 1.

Natural history of COPD

The probabilities of transitioning from one GOLD grade to the next were based on the pooled analysis by Hoogendoorn *et al*.[9] Progression in severity grades was assumed to be independent of the azithromycin treatment status because there is no evidence that azithromycin has a direct impact on lung function decline over time. Background mortality rates were taken from US life tables. Mortality directly related to exacerbations was exclusively modelled for severe and very severe events.[11]

Exacerbations were modelled as events, with annual rates modelled as a function of the underlying GOLD grades.[10] We chose the 'event-based' definition of exacerbations when extracting the related parameters from the literature and included moderate (requiring outpatient care or the initiation of antibiotics or systemic corticosteroids) and severe (requiring inpatient care) exacerbations.

Treatment effectiveness

Based on the MACRO study, a relative risk of 0.73 (0.63–0.84) was modelled for the effect of azithromycin.[4] In a sensitivity analysis, the effect size reported by a Cochrane systematic review that included other macrolides was considered.[12]

Treatment adverse events

We performed a scoping literature review to find the adverse events of azithromycin that are prevalent or consequential enough to materially affect its net benefit and to estimate the frequency of these events. All studies that evaluated the safety of azithromycin (in patients with COPD or other conditions) were considered. Our review identified the following adverse events: antimicrobial resistance, CV toxicity, hearing impairment and GI symptoms.

For antimicrobial resistance, the vast majority of studies compared the colonisation rate of macrolide-resistant bacteria in azithromycin users with that in control subjects.[12] A Cochrane

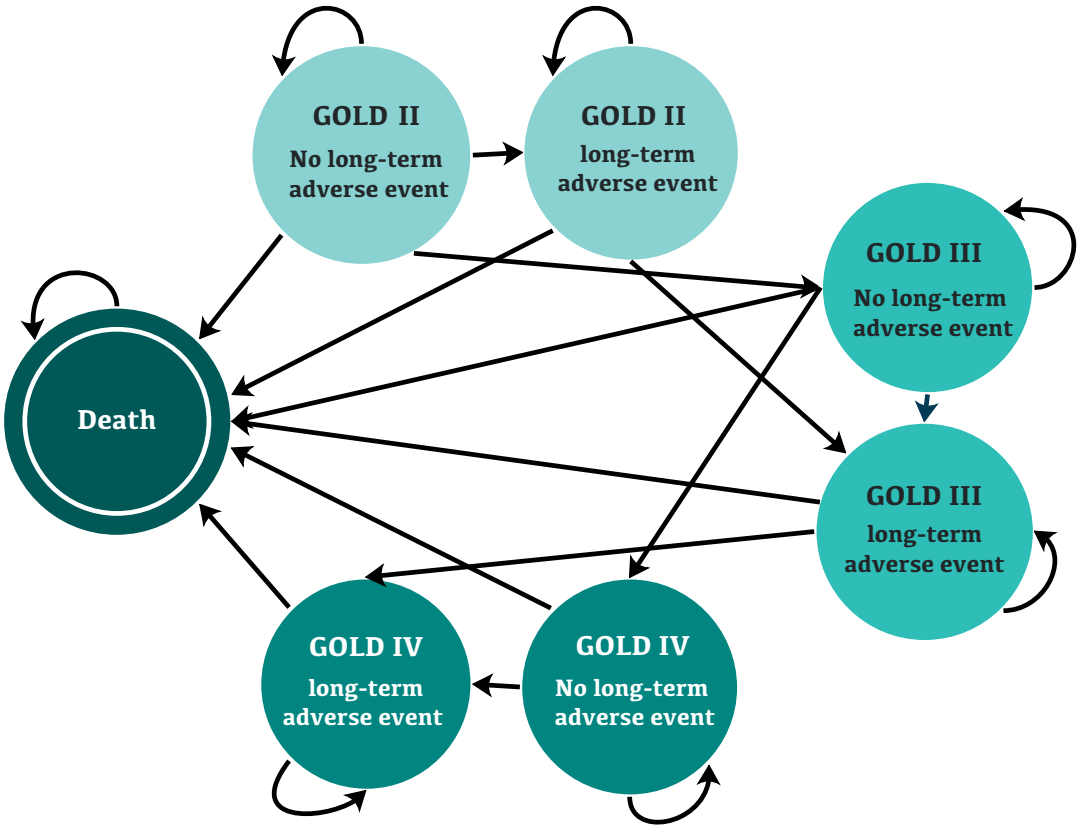

Figure 1 Schematic illustration of the model. GOLD[1] defines COPD as the ratio of FEV_1 to FVC of less than 0.7. The severity stages of the disease used in the model are defined as GOLD II (moderate COPD): 50%≤FEV_1<80% of a predicted reference value for a healthy individual; GOLD III (severe COPD): 30%≤FEV_1<50% predicted; and GOLD IV (very severe COPD): FEV_1<30% predicted. In the states with long-term adverse events, the exacerbation rate for each GOLD grade was the same as that reported by Hoogendoorn et al.[10]. Exacerbations tend to decrease in states without long-term adverse event because of the treatment effect. FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; COPD, chronic obstructive pulmonary disease.

Table 1 Input parameters of the model per person-year

	Base value			Probability distribution			Source
Parameter	**GOLD II**	**GOLD III**	**GOLD IV**	**GOLD II**	**GOLD III**	**GOLD IV**	
Initial distributions	0.26	0.4	0.34	NA	NA	NA	Albert *et al*[4]
Baseline age		65			NA		Albert *et al*[4]
Transition probability (95% CI)							Hoogendoorn *et al*[9]
Ex-smokers	0.034 (0.033 to 0.035)	0.030 (0.028 to 0.031)	NA	Beta (5703, 162 046)	Beta (1372, 44 377)	NA	
Current smokers	0.037 (0.036 to 0.038)	0.031 (0.029 to 0.032)	NA	Beta (6206, 161 543)	Beta (1418, 44 332)	NA	
Exacerbation rate in the reference group (95% CI)							Hoogendoorn *et al*[10]
Total exacerbations	1.17 (0.93 to 1.50)	1.61 (1.51 to 1.74)	2.10 (1.51 to 2.94)	Lognormal (0.16, 0.13)	Lognormal (0.48, 0.04)	Lognormal (0.74, 0.17)	
Severe exacerbations	0.16 (0.07 to 0.33)	0.22 (0.20 to 0.23)	0.28 (0.14 to 0.63)	Lognormal (–1.83, 0.37)	Lognormal (–1.51, 0.02)	Lognormal (–1.27, 0.40)	
Rate ratio of exacerbation (total and severe) by 12-month history pattern (95% CI)							ECLIPSE study[32] (online supplemental appendix 4)
No exacerbations in the first year		Total: 0.16 (0.14 to 0.17)			Total: normal (0.160, 0.007)		
		Severe: 0.16 (0.12 to 0.20)			Severe: normal (0.16, 0.02)		
≥1 moderate/severe exacerbations in the first year		1 (Reference)			NA		
≥2 moderate or ≥1 severe exacerbation in the first year		Total: 1.25 (1.16 to 1.33)			Normal (1.25, 0.04)		
		Severe: 1.36 (1.17 to 1.58)			Normal (1.36, 0.10)		
≥2 moderate/severe exacerbations in the first year		Total: 1.30 (1.20 to 1.40)			Normal (1.30, 0.05)		
		Severe: 1.26 (1.07 to 1.48)			Normal (1.26, 0.10)		
Relative risk of exacerbation in the treatment group compared with the control group (95% CI)							
Total		0.73 (0.63 to 0.84)			Lognormal (–0.31, 0.07)		Albert *et al*[4]
Ex-smokers		0.65 (0.55 to 0.77)			Lognormal (–0.43, 0.08)		Han *et al*[25]
Current smokers		0.99 (0.71 to 1.38)			Lognormal (–0.01, 0.17)		Han *et al*[25]
Decline in treatment effect		$RR_0^{\exp(-k*(year-1))}$ RR_0: first-year effect k=0.22			Fixed		Pomares *et al*[13] (online supplemental appendix 1)
Rate of mortality due to exacerbations (95% CI)		0.156 (0.109 to 0.203)			Beta (35.6, 192.4)		Hoogendoorn *et al*[11]
Hazard ratio of cardiovascular death in the first 5 days (95% CI)		2.88 (1.79 to 4.63)			Lognormal (1.06, 0.24)		Ray *et al*[6]
Relative risk of hearing loss due to azithromycin (95% CI)		1.168 (1.030 to 1.325)			Lognormal (0.15, 0.06)		Li *et al*[5]
Annual incidence of hearing loss (95% CI)		0.023 (0.011 to 0.035)			Normal (0.023, 0.006)		Lin *et al*[17] (online supplemental appendix 2)
Prevalence of GI symptoms in the population		0.332 (0.326 to 0.333)			Beta (28 945, 58 768)		Almario *et al*[18]
Relative risk of GI symptoms due to azithromycin (95% CI)		1.187 (0.761 to 1.849)			Lognormal (0.17, 0.22)		Li *et al*[5]
Baseline utility (EQ-5D) (95% CI)	0.787 (0.771 to 0.802)	0.750 (0.731 to 0.768)	0.647 (0.598 to 0.695)	Beta (2251, 609.4)	Beta (1666, 555.5)	Beta (245.7, 134.1)	Rutten-van Mölken *et al*[33]

Continued

Table 1 Continued

Parameter	Base value			Probability distribution			Source
	GOLD II	GOLD III	GOLD IV	GOLD II	GOLD III	GOLD IV	
Decrease in utility due to exacerbations (95% CI)							Sadatsafavi *et al*[34] (online supplemental appendix 3)
Mild and moderate	0.015 (0.002 to 0.040)	0.049 (0.020 to 0.090)	0.049 (0.020 to 0.090)	Beta (2.08, 132.50)	Beta (7.19, 140.30)	Beta (7.19, 140.30)	
Severe and very severe	0.068 (0.035 to 0.110)	0.065 (0.030 to 0.100)	0.065 (0.030 to 0.100)	Beta (11.92, 162.60)	Beta (12.7, 182.1)	Beta (12.7, 182.1)	
Decrease in utility due to hearing loss (95% CI)		0.187 (0.167 to 0.207)			Beta (6.92, 30.08)		NICE Guideline[21]
Utility improvement due to hearing aids (95% CI)		0.060 (0.044 to 0.073)			Normal (0.060, 0.006)		Barton *et al*[35]
Decrease in utility due to the GI symptoms (95% CI)		0.026 (0.024 to 0.028)			Beta (66 343, 2 475 535)		Sullivan and Ghushchyan[36] (online supplemental appendix 3)
Discount rate		3%			NA		Sanders *et al*[8]

ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; EQ-5D, EuroQol 5-Dimensional ; GI, gastrointestinal; GOLD, Global Initiative for Chronic Obstructive Lung Disease; NA, not applicable; NICE, National Institute for Health and Care Excellence.

systematic review reported that due to the variations in methodologies, the authors could not pool data on the colonisation rate and stated that there was insufficient evidence to predict how colonisation rates would affect the resistance patterns in the community.[12] Most empirical studies of preventive azithromycin have been short in duration. The longest study to date showed that azithromycin remained effective for at least 2 years; however, there was a 34% decline in its efficacy by year 3.[13] As such, in the base–case analysis, we modelled a gradual decline in the relative efficacy of azithromycin. We modelled a negative exponential decline in relative risk reduction for exacerbation that matched the observed decline in the third year of the aforementioned study (see online supplemental appendix 1).[13] This assumption was subjected to threshold analysis.

The major concern regarding CV toxicity of azithromycin is QT-interval prolongation and its associated sudden cardiac death.[6 14] A large US-based study demonstrated that in the first 5 days of exposure, the mortality risk increased by 288%,[6] but the risk was undetectable after 6 days.[15] In contrast, another study did not find an association between azithromycin and CV death.[16] To account for these findings, in the base–case analysis, we modelled an increase in mortality (due to sudden cardiac arrhythmia) immediately after initiation of therapy but no change in the mortality risk afterwards. This is overall a conservative assumption (against azithromycin), given the exclusion of patients with prolonged QTc interval in our study. Alternative assumptions were evaluated in the sensitivity and threshold analyses.

The added risk of hearing loss due to azithromycin was modelled on a relative scale as reported by Li *et al*,[5] applied to the background incidence of hearing loss taken from a population-based US study (see online supplemental appendix 2).[17] We assumed that the occurrence of new hearing impairment would result in the discontinuation of treatment and require the patients to wear hearing aids for the rest of their lives.

GI symptoms are known to be one of the adverse events of antibiotics[12] and have been reported in almost all of the trials studying the effect of azithromycin. Vomiting, abdominal pain, diarrhoea and decreased appetite were the most frequently recorded GI symptoms in patients who used azithromycin.[5] The background rate of GI symptoms in the general population was taken from a large (n=71 812) US study,[18] and the additional risk due to the treatment was derived from a systematic review by Li *et al* on the adverse events of azithromycin.[5] The GI symptoms were considered temporary and did not result in the discontinuation of treatment.

Health state utility values (utilities)

To calculate QALYs, each model state was assigned a utility value representing the average health-related quality of life of patients in that state. Short-term events (eg, exacerbations and GI symptoms) were modelled by an instantaneous drop in QALY. We used EuroQol 5-Dimensional (EQ-5D) utilities as the reference values,[19] given the large body of evidence regarding the quality of life across GOLD grades and the effect of exacerbations on the quality of life of patients measured using this instrument. See online supplemental appendix 3 for more details about how utilities were modelled.

However, it is noted that EQ-5D lacks sufficient sensitivity to detect reductions in quality of life due to hearing loss.[20] Therefore, we followed the approach adopted by the National Institute for Health and Care Excellence in its health technology assessment of early versus delayed management of hearing loss in adults,[21] and used the Health Utility Index (HUI)-3 instrument to capture changes in utility from hearing loss.[22] In a sensitivity analysis, we used EQ-5D utilities instead of HUI-3 for impaired hearing.

Analysis

The analysis was fully probabilistic, incorporating uncertainty in the input parameters to estimate net benefits through Monte Carlo simulation. Probability distributions were assigned based on the type of the parameter and the level of uncertainty (eg, width of CI). The primary benefit–harm metric was the net QALY gain from the use of azithromycin. Azithromycin was deemed to be net beneficial if the (average) expected value of QALY gained was positive. The probability of a positive gain in QALY was also quantified. Because the QALY gain is a random variable due to uncertainty in evidence, in line with previous studies,[23] we further required the probability of obtaining a positive QALY gain to be above 60% for azithromycin to be considered net beneficial. We did not report p values because in benefit–harm analysis, it is the overall expected change in the outcome of interest, independent of the level of statistical significance, that is relevant.[24]

Table 2 Outcomes of the probabilistic analysis over 20 years for 100 patients with COPD

	No azithromycin (95% CI)	Azithromycin (95% CI)	Difference (95% CI)
Total exacerbations (n)	2047 (1635 to 2567)	1931 (1545 to 2435)	−116 (−176 to −63)
Severe or very severe exacerbations (n)	280 (200 to 397)	265 (190 to 375)	−15 (−25 to −8)
Cumulative incidence of hearing loss (n)	24 (13 to 34)	28 (15 to 40)	4 (1 to 7)
Average episodes of GI symptoms (n)	19 (17 to 21)	23 (15 to 33)	4 (−3 to 13)
Mortality due to exacerbation (n)	40 (28 to 54)	38 (26 to 52)	−2 (−3 to −1)
Other mortality (Including CV death) (n)	34 (28 to 39)	35 (29 to 40)	1 (0 to 2)
Life years	1170 (1011 to 1303)	1204 (1053 to 1328)	34 (18 to 51)
Total QALYs	555.5 (482.7 to 624.4)	573.5 (504.7 to 637.8)	17.9 (6.2 to 30.0)

Results are reported for 100 patients.
GI, gastrointestinal; QALY, quality-adjusted life year.

Subgroup analysis

We investigated the benefit–harm of azithromycin among patients with different histories of exacerbations. We used individual-level data from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) cohort to calculate rate multipliers for each unique pattern of exacerbation in the previous 12 months (see online supplemental appendix 4). Furthermore, the potential benefit of treatment stratified by smoking status was explored using different effect sizes for former and current smokers, based on a subgroup analysis of the MACRO study.[25]

Threshold and sensitivity analyses

We performed separate threshold analyses to identify the threshold for each parameter at which the expected net benefit crossed zero. In addition, sensitivity analyses were conducted on CV death risk, treatment effect size, disutility due to hearing loss, discount rates, time horizon and adherence rates. Regarding adherence, given that parameters were derived from studies that used intention-to-treat analysis, our analysis incorporated loss in adherence over the treatment period. However, given that adherence to treatment in the community is generally lower than that in clinical studies, we also modelled the effect of permanent discontinuation of treatment (above and beyond treatment discontinuation in underlying RCTs) in a sensitivity analysis (online supplemental appendix 5).

All analyses were performed in R V.4.0.1. The open-source model and the analytical code are available online (https://github.com/safaahmadian/AZT_HarmBenefitAnalysis).

RESULTS

Table 2 provides the expected value of outcomes over 20 years for the main analysis, standardised to a cohort of 100 patients to facilitate interpretations. Among the primary target population (patients with COPD with GOLD grade ≥II with at least one moderate/severe exacerbation in the past 12 months), the treatment increased their QALYs by 17.9 per 100 patients over the given time horizon. The probability of positive expected QALY gain was 99.8%.

Subgroup analysis

The use of azithromycin in current smokers was associated with a net QALY loss of 4.3 per 100 patients over 20 years, and azithromycin was only 36.8% likely to be net beneficial. On the other hand,

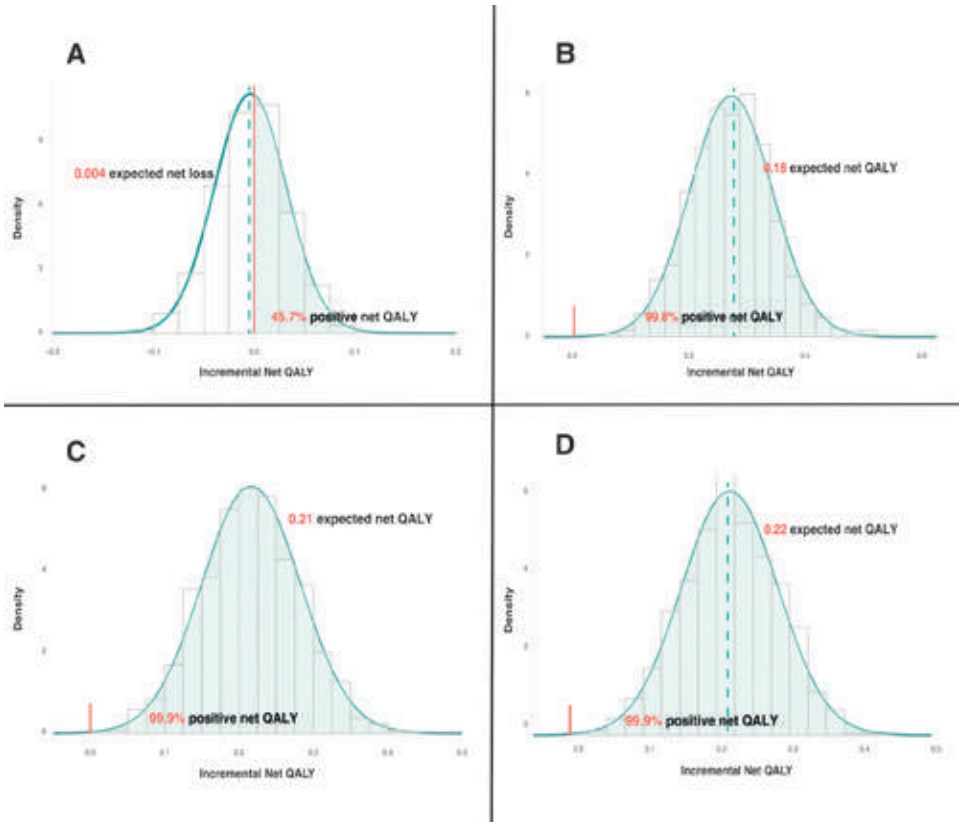

Figure 2 Probability distribution of net QALY gain according to patients' exacerbation history in the past 12 months. (A) Patients with no exacerbation; (B) patients with ≥1 moderate/severe exacerbations (the reference population); (C) patients with ≥2 moderate/severe exacerbations; (D) patients with ≥2 moderate or ≥1 severe exacerbations in the previous 12 months. QALY, quality-adjusted life years.

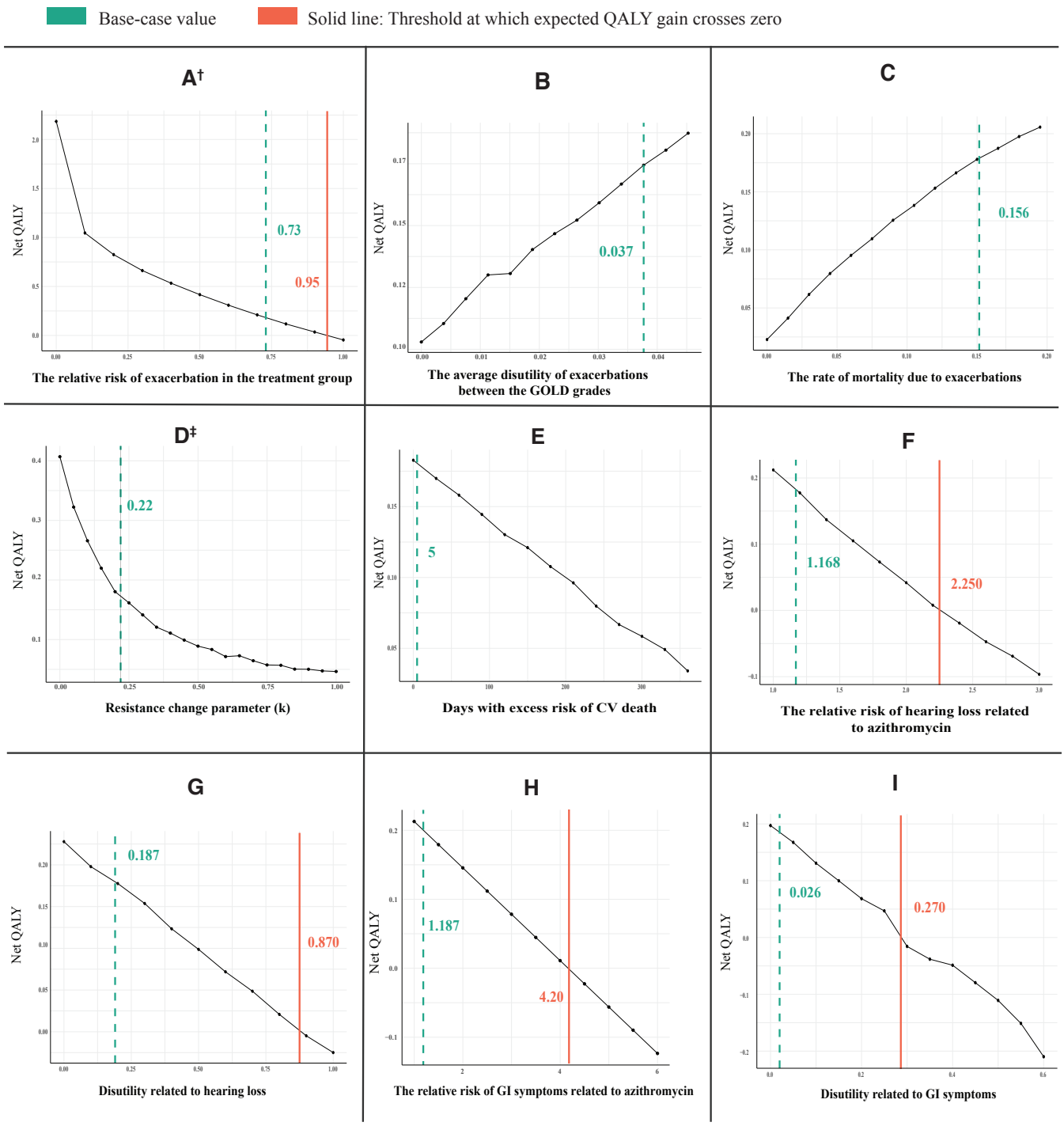

Figure 3 Results of threshold analyses. Each point on each graph is based on running the model for 1000 simulations.† The relative risk of exacerbation in the treatment group shows the treatment effect, ‡ The higher values of k in the equation, the faster the decline in the effectiveness of azithromycin over time. CV, cardiovascular; GI, gastrointestinal; GOLD, Global Initiative for Chronic Obstructive Lung Disease; QALY, quality-adjusted life years.

in ex-smokers, the expected net QALY gain was 25.3 per 100 patients over 20 years, and the probability of positive QALY gain was 99.9%.

Results of subgroup analysis by exacerbation history are provided in figure 2. Among patients with a negative exacerbation history in the previous 12 months, azithromycin was associated with an expected net QALY loss of 0.4 per 100 patients and a 45.7% chance of being net-beneficial, which did not meet the prespecified >60% probability criterion. Conversely, patients who had two or more moderate or at least one severe exacerbation in the previous year (the GOLD definition of a frequent exacerbator) derived the most benefit from azithromycin (expected net QALY of 21.8 per 100 patients, probability positive benefit=99.9%).

Threshold and sensitivity analyses

Figure 3 provides the results of the threshold analyses. In all figures, the solid line represents the threshold value at which the expected net benefit crosses zero. The value used in the base-case analysis is also highlighted by the dashed line for comparison.

Preventive azithromycin was net beneficial as long as it was associated with a risk reduction of 5% or more for exacerbations (figure 3A). Azithromycin remained net beneficial even if exacerbations were assumed not to cause a reduction in quality of life (figure 3B) or mortality (figure 3C), reflecting respectively, the survival benefit and gain in quality of life of preventing exacerbations. The net benefit of azithromycin approached zero only when the decline in the effectiveness of azithromycin was so high that azithromycin was only effective in the first year of treatment (figure 3D). Conversely, the net benefit was almost two times higher than the base-case value if the treatment effect did not wane over 20 years. Similarly, the assumption that the excess CV death risk persisted for 1 year did not change the direction of the expected net benefit (figure 3E). The relative risk of hearing loss and GI

symptoms should be 1.9 and 3.5 times higher than the base-case value, respectively, to result in a zero net benefit (figure 3F,H). Likewise, the reduction in quality of life due to hearing loss and GI symptoms should be implausibly high (0.87/year and 0.27/year, respectively) to nullify the net benefit gain (figure 3G,I).

Results of the sensitivity analyses are provided in the online supplemental appendix and show that the overall findings were robust to changes in several key assumptions in the analyses (no increased CV death risk, alternative source for treatment effect, smaller disutility due to hearing loss, discount rates of 0% and 5%, time horizon of 1 and 35 years, and explicit modelling of non-adherence to azithromycin).

DISCUSSION

We demonstrated that in patients with GOLD grade II or higher who have a positive exacerbation history despite using maximal inhaled therapies, show a normal QTc interval on baseline ECG and do not have comorbid asthma or existing hearing impairment, the addition of azithromycin is net beneficial. The probability of azithromycin conferring net benefit in such patients exceeded 90%. The expected gain in QALY (0.179) is within the range of QALY gain estimated for inhaled therapies from economic evaluations of such therapies (eg, 0.137 for triple vs double therapy and 0.131 between different dual therapies).[26 27] Our subgroup analyses showed that the benefit is mostly concentrated in patients who are not current smokers and who have had a positive exacerbation history in the previous 12 months. The GOLD management strategy,[1] the joint European Respiratory Society / American Thoracic Society (ATS/ERS),[28] and the statement by the American College of Chest Physicians/Canadian Thoracic Society[29] all recommend the use of azithromycin in patients who continue to exa-cerbate despite optimal inhaled therapies, but have also expressed concern about the adverse effects of maintenance azithromycin therapy. Our quantitative benefit–harm analysis provides further support for this recommendation, and our sensitivity and threshold analyses should lessen the concerns about the long-term balance of harms and benefits of this therapy. Importantly, given the high likelihood of net benefit of azithromycin in patients with only one moderate/severe exacerbation in the previous year (who also meet all the other inclusion criteria previously mentioned), our results support extending the eligibility criteria of 'frequent exacerbators' to include patients with any history of moderate/severe exacerbation in the previous year. It is noted that our results represent the benefit–harm balance of maintenance azithromycin therapy as evaluated in landmark clinical trials (eg, MACRO) and as recommended by contemporary disease management strategies. However, the manner in which azithromycin is prescribed in the 'real world' (eg, target population and adherence levels) might be different from guideline recommendations, and the actual net benefit of azithromycin can be affected by such departures from recommended usage.

Multiple sensitivity and threshold analyses provided assurances that our results were robust to a range of variations in the benefits and harms of azithromycin, including declining benefits of azithromycin on exacerbation risk over time, its potential to induce adverse events, and its impact on quality of life. Of particular concern among experts has been the durability of the net benefits of azithromycin over time, given the risk of antimicrobial resistance with its long-term use.[5] Some evidence suggests that treatment effect declines modestly over 3 years,[13] but to the best of our knowledge, empirical evidence beyond this period does not exist. Importantly, our results were robust to the assumption of diminishing treatment effects. These important findings indicate that concerns about the potentially diminishing effectiveness of azithromycin should not preclude its use in patients who still exacerbate despite optimal inhaled therapy.

To the best of our knowledge, this is the first quantitative benefit–harm analysis of maintenance azithromycin in COPD. Despite the relevance of quantitative benefit–harm analysis for evidence-inform decision-making, we are aware of only one benefit–harm analysis in the context of COPD that evaluated roflumilast.[30] Similar to our study, the authors collated data from multiple sources and combined multiple aspects of treatment using weights that reflected their clinical burden to derive a scalar net benefit index. They found that roflumilast was generally net harmful; the probability of it being net beneficial was >60% only among patients whose baseline risk of severe exacerbations was >22%/year. A difference between this study and ours is that the formerly assigned weights to different outcomes were based on expert opinion, whereas we used preference-based weights that gave rise to QALYs.

Our study has several strengths. The use of a probabilistic model enabled us to combine evidence from disparate sources to calculate a single index for the net benefit and to extrapolate the results to a sufficiently long time-horizon. By using health utility values as the 'currency' for quantifying harm and benefit, we were able to combine various aspects of benefit and harms and to generate a singular estimate of net benefit. Access to individual-level data from ECLIPSE enabled us to provide estimates of net benefit for subgroups based on their exacerbation history. By properly incorporating uncertainty in the evidence, we were able to make probabilistic statements about the likelihood of benefit, which showed that the evidence, while uncertain, points towards a high likelihood of net benefit in patients with a positive exacerbation history. This was further confirmed through multiple threshold and sensitivity analyses.

The limitations of this study should also be acknowledged. Given that in practice, azithromycin may be given for many years, the short-term follow-up time of RCTs provided a truncated picture of the overall benefits (or harms) of this treatment. As such, we had to extrapolate beyond the follow-up time of the longest empirical studies, which requires assumptions about the durability of benefits and harms. However, our sensitivity and threshold analyses showed that the conclusions do not change across a wide range of assumptions on the long-term trajectories of outcomes; in particular, our results remained robust against assumptions on the waning effect of azithromycin and the duration of heightened CV death risk. On the other hand, the effect of antimicrobial resistance in the community was not considered in this study and requires further investigation. We used utility weights from different sources as there was not a single study that consistently provided all the values required for our analysis, although we tried to reduce heterogeneity by prioritising values from the USA. Still, differences in the utility values for different model parameters might be affected by the differences in study design. Lastly, azithromycin is not the only choice of therapy in patients who exacerbate while on maximal inhaled therapy. However, because of an insufficient amount of direct evidence, we could not include other treatments (eg, roflumilast) in this analysis.

The model projected that azithromycin is not net beneficial in current smokers. The major source of evidence for the effect modification of smoking is a post hoc analysis of MACRO,[25] which may have been underpowered for this subgroup analysis (MACRO's sample size was based on identifying the main effect) and susceptible to a chance finding from multiple hypothesis testing. Observational studies have suggested that azithromycin is effective in reducing exacerbations in both current and ex-smokers, though the benefits were smaller in current than ex-smokers.[31] We were unable to use these data because of the possibility of confounding and bias, and in general, we did not find a properly designed quasi-experimental study that sufficiently adjusted for potential confounding variables. Studying the effectiveness of azithromycin in current smokers thus should be prioritised to generate high quality evidence to revisit this question. Further, we did not model the potential long-lasting effect of exacerbations (eg, drop in lung function), as we consider evidence on this aspect to still be controversial. However, we note that modelling such indirect treatment effect would lead to even greater benefits of the treatment and make our results even more favourable.

In summary, this study shows that azithromycin is very likely to be net beneficial in patients with COPD with a positive history of exacerbations. While the ATS/ERS statement has made a

conditional statement on the use of azithromycin in this patient subgroup and considered the quality of evidence to be low, our results suggest that this benefit is robust to many alternative assumptions. Future research should evaluate the effectiveness of azithromycin in current smokers. Quantitative benefit–harm analysis should also be considered for many other interventions in COPD for which the benefits and harms are only subjectively considered by expert panels.

Twitter Mohsen Sadatsafavi @mo_safavi
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ORCID iD
Mohsen Sadatsafavi http://orcid.org/0000-0002-0419-7862

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ARTIGO ORIGINAL

Precisão do Xpert Ultra no diagnóstico da tuberculose pediátrica num país com baixa incidência de tuberculose: um estudo multicêntrico prospetivo

David Aguilera-Alonso,[1,2,3,4] Gonzalo Solís-García,[5] Antoni Noguera-Julian,[3,6,7,8,9] Julián González-Martín,[4,10] Angely Román Cobeña,[10] Fernando Baquero-Artigao,[3,4,11] Carlos Toro Rueda,[12] Paula Rodríguez-Molino,[3,4,11] Iván Bloise Sánchez,[12] Teresa Vallmanya,[13] Albert Bernet-Sánchez,[14] Laura Minguell Domingo,[13] Adriana Rubio,[13] Jesús Saavedra-Lozano,[1,2,3,4,15] María Jesús Ruiz-Serrano,[16] Daniel Blázquez-Gamero,[3,15,17,18] Paula López-Roa,[19] David Gomez-Pastrana,[20] María Dolores López Prieto,[21] Eva María López Medina,[22] Ana Gil-Brusola,[23] Andrea Martín Nalda,[24] Antonio Soriano-Arandes,[24] Teresa Tórtola,[25,26] Lola Falcon-Neyra,[27,28] Verónica González Galán,[29] Marc Tebruegge,[30,31] Begoña Santiago-García[1,2,3,4]

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For numbered affiliations see end of article.

Correspondence to
Dr David Aguilera-Alonso, Department of Paediatrics, Hospital General Universitario Gregorio Marañón, Madrid, 28009, Spain; david.aguilera@salud.madrid.org

DA-A and GS-G are joint first authors.
MT and BS-G are joint senior authors.

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RESUMO

Introdução A tuberculose (TB) pulmonar infantil permanece um desafio de diagnóstico. Este estudo teve como objetivo avaliar o desempenho do Xpert Ultra no diagnóstico da tuberculose pulmonar em crianças num ambiente de baixa prevalência de tuberculose.

Métodos Estudo prospetivo, multicêntrico e de precisão diagnóstica. Foram recrutadas crianças com suspeita clínica ou radiológica de TB pulmonar em 11 unidades pediátricas em Espanha. Foram recolhidas até três amostras gástricas ou de expetoração em 3 dias consecutivos e analisadas por Xpert MTB/RIF, Xpert Ultra e cultura em paralelo.

Resultados Foram incluídas 86 crianças (idade média de 4,9 anos, IQR 2,0-10,0; 51,2% do sexo masculino). O diagnóstico final foi TB pulmonar em 75 pacientes (87,2%); 33 (44,0%) foram microbiologicamente confirmados. Foi analisado um total de 219 amostras, incluindo aspirados gástricos (n=194; 88,6%) e amostras de expetoração (n=25; 11,4%). Recorrendo à cultura como padrão de referência e comparando amostras individuais, a sensibilidade foi de 37,8% (14/37) para o Xpert MTB/RIF e de 81,1% (30/37) para o Xpert Ultra (p<0,001); a especificidade foi de 98,4% (179/182) e de 93,4% (170/182), respetivamente (p=0,02). Na análise por paciente, considerando os resultados positivos em qualquer amostra, a sensibilidade foi de 42,9% (9/21) para Xpert MTB/RIF e de 81,0% para Xpert Ultra (17/21, p=0,01); a especificidade foi de 96,9% (63/65) e de 87,7% (57/65, p=0,07), respetivamente.

Conclusões Em crianças com tuberculose pulmonar num cenário de baixa incidência, o Xpert Ultra possui uma sensibilidade significativamente mais elevada do que a geração anterior do teste Xpert e apenas uma especificidade marginalmente mais baixa. Assim, em crianças sob avaliação por suspeita de TB pulmonar, deve ser usado o Xpert Ultra em vez do Xpert MTB/RIF sempre que possível.

Key messages

WHAT IS ALREADY KNOWN ON THIS TOPIC

► Xpert Ultra in children in countries with a high tuberculosis (TB) burden has shown a higher sensitivity but slightly lower specificity than the previous generation assay, Xpert MTB/RIF assay.

WHAT THIS STUDY ADDS

► In children with pulmonary TB in a low burden setting, Xpert Ultra has significantly higher sensitivity than the previous generation assay and only marginally lower specificity.

► In addition, Xpert Ultra produced positive assay results in a considerable number of culture-negative samples, suggesting that this assay may have greater sensitivity than mycobacterial culture.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

► In children undergoing evaluation for suspected pulmonary TB, Xpert Ultra should be used in preference to Xpert MTB/RIF whenever possible.

INTRODUCTION

Globally, tuberculosis (TB) is the leading cause of death by a single infectious agent. In 2020, an estimated 10 million cases and 1.5 million deaths occurred due to TB, 11% of which were in children.[1] However, there are data suggesting that the true incidence of TB in children is threefold higher than the official notification figures indicate.[2] Children are more likely than adults to have TB disease with low bacterial loads (ie, paucibacillary disease), with resulting culture yields as low as 25%–40%.[3]

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, California, USA) has been a notable advance in diagnosing TB. The assay is able to detect *Mycobacterium tuberculosis* complex (MTB), and simultaneously genotypic rifampicin resistance, with an estimated limit of detection of 114 colony forming units (CFU)/mL. Xpert MTB/RIF's pooled sensitivity in smear-positive adults with pulmonary TB is 98%, with a pooled specificity of 98%. However, the sensitivity in smear-negative adults, compared with culture, has been estimated to be only 67%.[4] A meta-analysis conducted by the WHO estimated that in children, the pooled sensitivity of Xpert MTB/RIF compared with culture was approximately 65%.[5] Nevertheless, a more recent meta-analysis estimated the pooled sensitivity in

children<4 years of age to be as low as 44% and 66% in children aged 5–15 years, indicating that Xpert MTB/RIF is unsuitable as a rule-out test in children with suspected TB.[6]

A new generation Xpert assay, Xpert MTB/RIF Ultra (Xpert Ultra), was subsequently developed to overcome the limited sensitivity of Xpert MTB/RIF in paucibacillary disease. Compared with the previous generation, the Xpert Ultra assay has two additional multicopy amplification targets for MTB (IS6110 and IS081), resulting in a lower detection limit of 16 CFU/mL. The specificity of rifampicin resistance testing has also been improved by implementing a melting temperature–based analysis.[7] The Xpert Ultra assay also provides a new semiquantitative categorical result called 'trace'. Because of the typically low bacterial load in paediatric TB, the WHO recommends interpreting trace results as positive in children.[8]

Recent studies have evaluated the performance of Xpert Ultra in children in countries with a high TB burden, such as South Africa, Tanzania, China and India, showing higher sensitivity but slightly lower specificity than the previous generation assay.[9–14] Findings of studies assessing TB diagnostic tests can vary greatly depending on local TB transmission and sociodemographic characteristics of the study population. However, to date, no prospective studies on the assay's performance in settings with low TB prevalence have been published.[7] To our knowledge, this is the first study aiming to evaluate the performance of Xpert Ultra for the diagnosis of pulmonary TB in children in a country with low TB prevalence.

METHODS

Study design and participants

We performed a prospective, multicentre, diagnostic accuracy study. Eleven tertiary referral hospitals that are part of the Spanish Paediatric TB Research Network (pTBred)[15] participated in the study. Eligible for participation were children and adolescents below 18 years of age with clinically suspected pulmonary TB, based on clinical signs and symptoms (online supplemental appendix p1), and/or a chest X-ray classified as 'consistent with TB', as per published consensus criteria (online supplemental appendix p2).[16 17] Patients were excluded if they had received anti-TB treatment in the previous 6 months or if none of the patient's specimens were tested with Xpert Ultra and Xpert MTB/RIF in parallel. The recruitment period was from 1 January 2018 to 30 September 2020.

Study protocol, data collection and study definitions

The study data were collected in the established pTBred database, using REDCap electronic data capture tools hosted at the Gregorio Marañón Hospital.[18] Demographic information, medical history, chest imaging results, HIV status and clinical data were recorded at enrolment.

Children were categorised into three subgroups, based on published consensus criteria[16]: (1) confirmed TB: a child with at least one respiratory specimen (ie, gastric aspirate or sputum) positive for MTB in culture or PCR; (2) unconfirmed TB: a child without bacteriological confirmation of MTB who met at least 1 (if immunological evidence of MTB present) or 2 (if immunological evidence of MTB absent) of the following criteria: symptoms/signs suggestive of TB, chest radiograph consistent with TB, close TB exposure or positive clinical response to TB treatment; (3) unlikely TB: a child in whom MTB was not detected, in combination with an alternative diagnosis and clinical recovery without anti-TB treatment.

Immunological evidence of MTB infection was defined as a positive tuberculin skin test (TST) and/or interferon-gamma release assay (IGRA) result. TSTs were performed by intradermal injection of two tuberculin units of purified protein derivative (PPD RT23; Statens Serum Institut, Copenhagen, Denmark). According to the Spanish National TB Guidelines, TST was considered positive if the induration at the injection site was ≥5 mm at 48–72 hours.[19] At least one specialised paediatric radiologist evaluated the chest X-ray(s) of each participant. TB treatment was initiated at the discretion of the treating physician. Microbiological results and response to treatment were regularly assessed during follow-up.

Microbiological procedures

Before initiating TB treatment, respiratory specimens were obtained in the early morning on up to 3 consecutive days. The treating physician decided on the type of specimen to be collected, depending on the child's ability to expectorate sputum: gastric aspirate, induced sputum or spontaneous sputum. The method for obtaining an induced sputum or gastric lavage specimen is included in the online supplemental appendix p3. In some patients, additional specimens were collected at the discretion of the treating physician. Gastric aspirates were neutralised with phosphate buffered saline, and all specimens were decontaminated with sodium hydroxide-N-acetyl-L-cysteine. After centrifugation, the supernatant was discarded, and the sediment was resuspended in 2.5 mL of phosphate buffered saline solution. From that suspension, ≥0.8 mL was used to prepare smears for microscopic examination, liquid and solid culture. Finally, two aliquots of ≥0.7 mL were separated for Xpert MTB/RIF and Xpert Ultra assays. For Xpert assays, sample reagent was added in a 2:1 dilution. The resulting samples were transferred into Xpert cartridges, inserted into the test platform (GeneXpert system, Cepheid) and processed according to the manufacturer's instructions. The semiquantitative scale for Xpert Ultra results comprised 'trace', 'very low', 'low', 'medium' or 'high', and for Xpert MTB/RIF 'very low', 'low', 'medium' or 'high'. If Xpert Ultra result is 'trace', rifampicin susceptibility is not evaluated. In the remaining positive Xpert MTB/RIF or Ultra results, rifampicin susceptibility is evaluated according to the manufacturer's instructions. Liquid cultures were incubated for up to 6 weeks and solid medium cultures for up to 8 weeks. In children with a positive mycobacterial culture, phenotypic drug susceptibility was determined using the proportion method recommended by WHO.[20] Microbiological tests were carried out on fresh clinical specimens, except in five participants during a shortage of Xpert cartridges, when specimens had to be frozen at −80°C for later analysis. A summary of the microbiological techniques used in this study is provided in the online supplemental appendix p4 and p5.

Statistical analysis

The performance of Xpert MTB/RIF and Xpert Ultra assays was compared against two reference standards: (1) a culture-based reference standard (solid or liquid culture positive for MTB from a respiratory specimen), and (2) a clinical reference standard (children with confirmed or unconfirmed pulmonary TB, as described above). Further, the diagnostic accuracy of both tests was analysed and compared by two modalities: (1) per-patient analysis: the assay performance was compared across patients, who were considered 'positive' if they had at least one specimen with a positive result; and (2) per-specimen analysis: the assay performance was compared across all individual specimens.

Continuous variables were described as medians and IQRs as the data were non-normally distributed, and categorical variables as number and percentages. The sensitivity and specificity were calculated with Clopper-Pearson 95% CIs, and the receiver operating characteristic curve (ROC) and the area under the ROC (AUROC) with DeLong 95% CI. McNemar's test was used to evaluate differences in sensitivity and specificity between Xpert MTB/RIF and Xpert Ultra. A p value<0.05 was considered statistically significant.

We calculated the required sample size to compare diagnostic tests using paired groups. Based on a previous study using respiratory specimens from children, the absolute difference in sensitivity between Xpert Ultra and Xpert MTB/RIF was expected to be 24% using culture as the reference standard.[21] Under the assumption that approximately 40% of the children with suspected TB would have a positive culture result,[3] we estimated that a sample size of 170 patients was required, with a power of 0.80 and an α of 0.05 in the per-patient analysis. SPSS V.23.0 software (SPSS Statistics, IBM Corporation) was used for statistical analysis and Epidat V.4.1 software (Xunta de Galicia, Spain) for sample size calculation. STARD 2015 guidelines for reporting diagnostic accuracy studies were followed throughout.[22]

RESULTS

The study was closed before the calculated sample size was reached. Recruitment had been slower than anticipated, likely due to a combination of declining TB incidence in Spain, and, more importantly, the strain the COVID-19 pandemic had put on clinical and research staff. Following an interim analysis that indicated that sufficient patient numbers for the main hypothesis had been met (superior sensitivity of Xpert Ultra compared with Xpert MTB/RIF), a decision was made to terminate the study early.

A total of 102 patients were recruited during the study period. Sixteen patients were excluded (figure 1), and, therefore, 86 patients and 219 corresponding specimens were included in the final analysis. Fifty-seven patients (66.3%) had three specimens collected, 19 (22.1%) two specimens and 10 (11.6%) one specimen.

The median age was 4.9 years (IQR 2.0–10.0), and 51.2% were male. None of the patients had a previous diagnosis of TB, and none had known HIV infection. Sixty-four patients (74.4%) had clinical signs or symptoms suggestive of pulmonary TB, while 57 (66.2%) had radiological findings consistent with TB. The most common symptom reported was cough (n=37; 43.0%), followed by fever >38.0°C (n=34; 39.5%). The clinical and epidemiological characteristics at presentation and radiological findings are summarised in tables 1 and 2.

Among the 86 patients included in the analysis, the final diagnosis was pulmonary TB in 75 (87.2%), of whom 33 (44.0%) had microbiologically confirmed TB. The remaining 11 (12.8%) patients were categorised as unlikely TB.

Twenty-one patients (24.4%) had at least one sample that grew MTB in culture, 11 (12.8%) had at least one specimen with a positive Xpert MTB/RIF result and 25 (29.1%) had at least one specimen with a positive Xpert Ultra result. Only two patients (2.3%) had a positive smear. No case of rifampicin resistance was identified by either phenotypic susceptibility testing or Xpert MTB/RIF assays.

Of the 219 analysed specimens, 194 (88.6%) were gastric aspirates and 25 (11.4%) were sputum specimens (n=13 spontaneous and n=12 induced sputum). Five specimens (2.3%) were positive by smear microscopy, MTB grew in culture in 37 (16.9%) specimens, Xpert MTB/RIF was positive in 17 (7.8%) and Xpert Ultra was positive in 42 (19.2%). The agreement between the three tests is summarised in figure 2. The semiquantitative results of Xpert MTB/RIF and Xpert Ultra are shown in table 3.

Per-patient analysis

The per-patient analysis is summarised in table 4. Considering a positive culture result in any specimen as the reference standard, the sensitivity was 42.9% for Xpert MTB/RIF and 81.0% for Xpert Ultra (p=0.01), while the assay specificities were 96.9% and 87.7%, respectively (p=0.07). The AUROC of Xpert MTB/RIF and Xpert Ultra were 0.699 (95% CI 0.588 to 0.809) and 0.843 (95% CI 0.748 to 0.938), respectively (online supplemental appendix figures 1 and 2). Including only symptomatic patients (n=64) in the analysis, the sensitivity and specificity estimates were similar to those observed in the entire cohort (online supplemental appendix table 1). Two patients with TB were Xpert MTB/RIF-positive and culture-negative, and eight were Xpert Ultra-positive and culture-negative.

When using the composite clinical reference standard, the sensitivity was 13.3% for Xpert MTB/RIF, 34.7% for Xpert Ultra (p<0.001 compared with Xpert MTB/RIF) and 28.0% for culture (21/75, 95% CI 18.2 to 39.6, p=0.267 compared with Xpert Ultra), with all three tests showing a specificity of 100% (11/11).

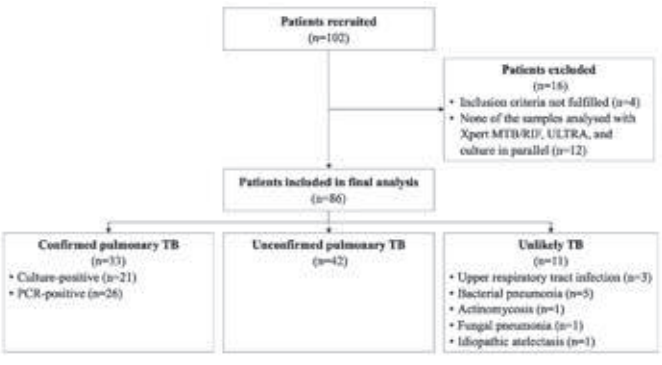

Figure 1 Flow chart summarising participant recruitment and classification into diagnostic subgroups. TB, tuberculosis.

Table 1 Summary of epidemiological and clinical characteristics and test results in the study population according to diagnostic subgroup

	Pulmonary TB (n=75)	Unlikely TB (n=11)
Age, years	4.3 (2.0–9.4)	9.0 (3.0–13.0)
Gender, male	37/75 (49.3)	7/11 (63.6)
Reason for TB screening		
Contact tracing	40/75 (53.3)	2/11 (53.3)
Clinical signs and/or symptoms of TB	39/75 (52.0)	7/11 (50.7)
Migrant screening	4/75 (5.3)	1/11 (5.3)
Radiological findings	0/75 (0)	1/11 (5.3)
Tuberculin skin test result*		
Positive	61/66 (92.4)	2/10 (20.0)
Negative	5/66 (7.6)	8/10 (80.0)
QFT result†		
Positive	51/58 (87.9)	0/11 (0)
Negative	6/58 (10.3)	10/11 (81.8)
Indeterminate	1/58 (1.7)	1/11 (9.1)
Clinical symptoms		
Asymptomatic	20/75 (26.7)	2/11 (18.2)
Cough	31/75 (41.3)	6/11 (54.5)
Fever	29/75 (38.7)	5/11 (45.5)
Asthenia	16/75 (21.3)	3/11 (27.3)
Weight loss	15/75 (20.0)	1/11 (9.1)
Vomiting	9/75 (12.0)	0/11 (0)
Other GI symptoms	8/75 (10.7)	0/11 (0)
Respiratory distress	4/75 (5.3)	3/11 (27.3)

Data are shown as number (percentage) and median (IQR).
*Positive cut-off: ≥5 mm induration. TST results were unknown in nine children with pulmonary TB and one child with unlikely TB.
†QuantiFERON-TB Gold assay results were unknown in 2 children with pulmonary TB; in 15 children with pulmonary TB, the test was not performed.
GI, gastrointestinal; QFT, QuantiFERON-TB assay; TB, tuberculosis.

Per-specimen analysis

A summary of the per-specimen analysis is shown in table 4. Using culture as the reference standard and comparing individual specimens, the sensitivity of Xpert MTB/RIF was 37.8% and that of Xpert Ultra was 81.1% (p<0.001), while the specificity was 98.4% and 93.4%, respectively (p=0.02). The AUROC of Xpert MTB/RIF and Xpert Ultra were 0.681 (95% CI 0.601 to 0.761) and 0.872 (95% CI 0.806 to 0.939), respectively (online supplemental appendix figures 3 and 4). Three specimens were Xpert MTB/RIF-positive and culture-negative, and 12 were Xpert Ultra-positive and culture-negative.

Using the composite clinical reference standard, sensitivity was 9.1% for Xpert MTB/RIF, 22.5% for Xpert Ultra (p<0.001 compared with Xpert MTB/RIF) and 19.8% for culture (p=0.36 compared with Xpert Ultra). Based on a total of 32 specimens from 11 patients with unlikely TB, specificity was 100% (32/32) for Xpert MTB/RIF, Xpert Ultra and culture. Stratified sensitivity and specificity according to specimen type (gastric aspirate or sputum) are detailed in the online supplemental appendix tables 2 and 3; sensitivity and specificity stratified by age are shown in the online supplemental appendix tables 4 and 5.

Table 2 Summary of radiological findings in the study population according to diagnostic subgroup

	Pulmonary TB (n=75)	Unlikely TB (n=11)
Chest X-ray findings	n=75	n=11
No abnormalities detected	26 (34.7)	3 (27.3)
Hilar lymphadenopathy	26 (52.0)	1 (9.1)
Parenchymal opacities	25 (5.3)	5 (45.5)
Pleural effusion	12 (16.0)	3 (27.3)
Airway stenosis/segmental atelectasis	7 (9.3)	1 (9.1)
Paratracheal lymphadenopathy	5 (6.7)	0 (0)
Pulmonary cavitation	3 (4.0)	1 (9.1)
Subcarinal lymphadenopathy	2 (2.7)	0 (0)
Pulmonary calcifications	1 (1.3)	1 (9.1)
CT findings	n=48	n=6
Hilar lymphadenopathy	33 (68.8)	3 (50.0)
Parenchymal opacities	32 (66.7)	5 (83.3)
Paratracheal lymphadenopathy	16 (33.3)	2 (33.3)
Pulmonary calcifications	12 (25.0)	1 (16.7)
Airway stenosis/atelectasis	9 (18.8)	1 (16.7)
Pleural effusion	9 (18.8)	3 (50.0)
Pulmonary cavitation	5 (10.4)	1 (16.7)
Miliary opacities	1 (2.1)	0 (0)

Data are shown as number (percentage) and median (IQR).
TB, tuberculosis.

The 12 respiratory specimens that were Xpert Ultra-positive and culture-negative originated from 11 children. Of those, three were concordantly positive in culture and Xpert Ultra in other non-respiratory specimens, while eight had negative culture, Xpert MTB/RIF and Xpert Ultra results in their additional non-respiratory specimens. Of the Xpert Ultra-positive/culture-negative specimens, 10 (83.3%) had a semiquantitative result of 'trace', 1 (8.3%) 'very low' and 1 (8.3%) low. Only one (9.1%) of those specimens tested positive in the Xpert MTB/RIF assay. All 11 children with Xpert Ultra-positive/culture-negative results responded to TB treatment and were considered cured by their attending physician at the end of treatment. Additional data on those patients are shown in the online supplemental appendix table 6.

DISCUSSION

To our knowledge, this is the first prospective multicentre study evaluating the performance of the Xpert Ultra assay in children with suspected pulmonary TB in a low TB prevalence setting. Also, it is one of a small number of paediatric studies that have included a three-way head-to-head comparison between Xpert Ultra, Xpert

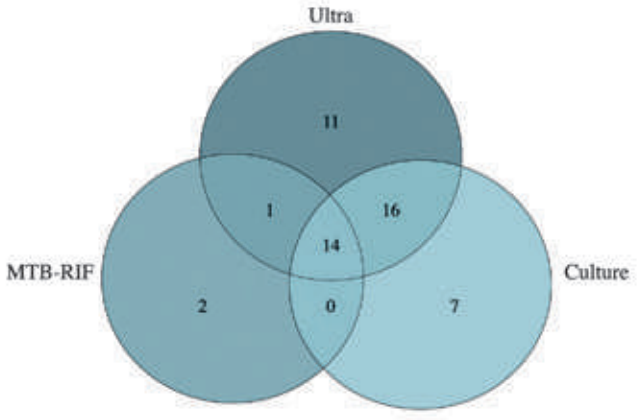

Figure 2 Venn diagram summarising positive Xpert Ultra, Xpert MTB/RIF and mycobacterial culture results according to per-sample analysis.

Table 3 Semiquantitative results of Xpert MTB/RIF and Xpert Ultra assays in all clinical specimens analysed (n=219)

Xpert MTB/RIF		Xpert Ultra	
Total negative	202 (92.2)	Total negative	177 (80.8)
Total positive	17 (7.8)	Total positive	42 (19.2)
High	1 (0.5)	High	1 (0.5)
Medium	2 (0.9)	Medium	2 (0.9)
Low	5 (2.3)	Low	11 (5.0)
Very low	9 (4.1)	Very low	9 (4.1)
–	–	Trace	19 (8.7)

Data are shown as number (percentage).

MTB/RIF and mycobacterial culture. A further key strength of our study lies in the use of fresh clinical specimens, with a few exceptions. Notably, the first two published reports on Xpert Ultra in children used frozen specimens that had been stored for an extended period of time,[9 10] and the resulting estimates may therefore not reflect the assay's true performance in a real-life clinical setting.

Both the per-patient and the per-specimen analysis, using positive culture results as the reference standard, showed that Xpert Ultra had significantly higher sensitivity than the previous generation assay, Xpert MTB/RIF, in our cohort (81.1% vs 37.8% and 81.0% vs 42.9%, respectively). As is generally the case with diagnostic assays, the trade-off for enhanced sensitivity was a small, but statistically significant, reduction in assay specificity (93.4% vs 98.4% in per-specimen analysis, respectively). Particularly in the paediatric setting, where paucibacillary disease is common and patients are at greater risk of severe and disseminated TB disease than adults, the improved assay sensitivity is of far greater importance than this minor reduction in specificity. Consequently, Xpert Ultra should be used, in preference to Xpert MTB/RIF, in the diagnostic workup of children with suspected TB.

A number of previous studies have evaluated Xpert Ultra in adults in a variety of settings.[7] The largest to date, which compared Xpert MTB/RIF and Xpert Ultra in adults with clinically suspected TB, included 1753 patients recruited at 10 sites in 8 low-income and middle-income countries.[23] Dorman *et al* reported that overall, the sensitivity of Xpert Ultra was 5.4% higher, and its specificity 2.7% lower than that of Xpert MTB/RIF. However, a far greater gain in sensitivity was observed in the subgroup of patients who were smear-negative, from 46.0% to 63.0%. Based on this observation, the authors hypothesised that children, who frequently have smear-negative disease, could benefit the most from the new assay, which is supported by our findings. Another prospective adult study conducted in Switzerland, a low TB prevalence setting, showed similar results as the aforementioned report.[24]

Studies comparing Xpert MTB/RIF and Xpert Ultra in children in high TB prevalence settings, including sub-Saharan Africa and the Indian subcontinent, have also reported enhanced sensitivity of Xpert Ultra, ranging from 5% to 13%.[9–14] In our study, the increase in sensitivity between both assays was even greater (38.1% in the per-patient analysis) when using culture as the reference standard. One possible explanation is that our patients may have presented earlier at a less advanced disease stage with lower bacterial loads, considering that the number of smear-positive cases in our study was very low (only 2/86 (2.3%) patients), a setting where the lower detection limit of the Xpert Ultra is likely to have the greatest impact. Another possible explanation for the comparatively high sensitivity of Xpert Ultra in our cohort could be related to the fact that fresh clinical specimens were used, while many previous studies tested frozen specimens. A retrospective study in children in Italy solely using gastric specimens compared the sensitivity of Xpert Ultra and Xpert MTB/RIF against mycobacterial culture, reporting test sensitivities of 84.2% and 45.5%, respectively. An additional limitation of that study is that the test sensitivities were only compared indirectly, as the tests were performed on different clinical specimens.[25]

Table 4 Per-specimen and per-patient analysis of the sensitivity and specificity of Xpert Ultra and Xpert MTB/RIF

Per-patient analysis (n=86)						
	Sensitivity			Specificity		
Reference standard	Xpert MTB/RIF	Xpert Ultra	P value	Xpert MTB/RIF	Xpert Ultra	P value
Culture	42.9% (9/21)	81.0% (17/21)	0.01	96.9% (63/65)	87.7% (57/65)	0.07
	95% CI 21.8 to 66.0	95% CI 58.1 to 94.6		95% CI 89.3 to 99.6	95% CI 77.2 to 94.5	
Clinical diagnosis	13.3% (10/75)	34.7% (26/75)	<0.001	100% (11/11)	100% (11/11)	0.99
	95% CI 6.7 to 21.3	95% CI 24.0 to 46.5		95% CI 70.0 to 100*	95% CI 70.0 to 100*	
Per-specimen analysis (n=219)						
	Sensitivity			Specificity		
Reference standard	Xpert MTB/RIF	Xpert Ultra	P value	Xpert MTB/RIF	Xpert Ultra	P value
Culture	37.8% (14/37)	81.1% (30/37)	<0.001	98.4% (179/182)	93.4% (170/182)	0.02
	95% CI 22.5 to 55.2	95% CI 64.8 to 92.0		95% CI 95.3 to 99.7	95% CI 89.8 to 96.5	
Clinical diagnosis	9.1% (17/187)	22.5% (42/187)	<0.001	100% (32/32) 95%	100% (32/32)	0.99
	95% CI 5.4 to 14.2	95% CI 16.7 to 29.1		95% CI 87.3 to 100*	95% CI 87.3 to 100*	

*95% CI calculated with the modified Wald method.

There is ongoing debate whether the use of mycobacterial cultures as the microbiological gold standard for pulmonary TB remains justified, considering that the performance of molecular tests is improving continuously and may have already surpassed culture.[26] We found that while Xpert Ultra detected MTB in 42 specimens, only 37 specimens showed positive culture results. Overall, there were 12 specimens from 11 patients with an Xpert Ultra-positive/culture-negative result in respiratory specimens. All but one of those patients had immunological evidence of exposure to MTB (n=8 TST-positive; n=8 IGRA-positive), nine had radiological findings consistent with TB, and all showed clinical response to anti-TB treatment. This suggests that those patients likely had TB disease and, therefore, a true-positive Xpert Ultra and a false-negative culture result. If this is the case, in our cohort, culture identified 21 of the confirmed TB cases while Xpert Ultra identified an additional 8 cases, representing an increase of 38.1% in the detection rate. Those observations indicate that combining culture with Xpert Ultra in each specimen can not only accelerate the diagnostic process, but may potentially increase the diagnostic yield substantially. Notably, all specimens from those 11 patients had a semiquantitative Xpert Ultra result of 'very low' or 'trace', which indicates that the mycobacterial load present in those specimens was low.

Studies focused on extrapulmonary TB in children, such as TB meningitis, have highlighted the limitations of microbiological diagnostic tools.[27] One study in adults with TB meningitis has reported that Xpert Ultra has substantial higher sensitivity than both Xpert MTB/RIF and culture in cerebrospinal fluid samples.[28] Those data are encouraging, but further data specifically in children are needed. Recent data also suggest that Xpert Ultra performed on stool children with pulmonary TB has higher sensitivity than Xpert MTB/RIF.[29]

Our study has some limitations. First, in accordance with most TB diagnostic studies, we used culture as the gold standard, which may be suboptimal, as discussed above. Notably, it has been estimated that culture-confirmation of childhood TB disease is achieved in less than 40% of cases.[1] To address this issue, we also evaluated the performance of the assays against a composite clini-cal reference standard. Furthermore, the number of patients with TB disease included in this study was limited, despite involving eleven tertiary referral centres for paediatric TB, which reflects the steady decline in TB incidence in Spain over the last decade.[1] Despite this, the study had sufficient statistical power to detect significant differences between the performance of Xpert MTB/RIF and Xpert Ultra.

In conclusion, our data show that, in children with pulmonary TB, Xpert Ultra has significantly higher sensitivity than Xpert MTB/RIF in a low TB prevalence setting. Furthermore, almost a third of the confirmed TB cases had a positive Xpert Ultra result but a negative culture result, highlighting that the combined use of both tests may increase the diagnostic yield substantially. However, when using a composite clinical reference standard, we found that in more than half of the TB cases in our cohort microbiological confirmation was not achieved, underscoring the ongoing need to develop better diagnostic tools for paediatric TB.

Author affiliations
[1]Paediatric Infectious Diseases Unit, Hospital General Universitario Gregorio Marañón, Madrid, Spain
[2]Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid, Spain
[3]Translational Research Network in Paediatric Infectious Diseases (RITIP), Madrid, Spain
[4]Centro de Investigación Biomedica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain
[5]Neonatology Department, The Hospital for Sick Children, Toronto, Ontario, Canada
[6]Malalties Infeccioses i Resposta Inflamatòria Sistèmica en Pediatria, Unitat d'Infeccions, Servei de Pediatria, Hospital Sant Joan de Deu, Barcelona, Spain
[7]Institut de Recerca Pediàtrica Hospital Sant Joan de Déu, Barcelona, Spain
[8]Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[9]Department of Paediatrics, University of Barcelona, Barcelona, Spain
[10]Servei de Microbiologia, Hospital Clinic de Barcelona, Barcelona, Spain
[11]Department of Paediatrics, Infectious, and Tropical Diseases, Hospital La Paz, Madrid, Spain
[12]Department of Microbiology, Hospital La Paz, Madrid, Spain
[13]Department of Paediatrics, Hospital Universitari Arnau de Vilanova, Lleida, Spain
[14]Laboratory of Microbiology, Hospital Universitari Arnau de Vilanova, Lleida, Spain
[15]Universidad Complutense, Madrid, Spain
[16]Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain
[17]Paediatric Infectious Diseases Unit, Hospital Universitario 12 de Octubre, Madrid, Spain
[18]Instituto de Investigación Hospital 12 de Octubre (imas12), Madrid, Spain
[19]Department of Microbiology, Hospital Universitario 12 de Octubre, Madrid, Spain
[20]Paediatric Respiratory Unit, Hospital Universitario Materno-Infantil de Jerez, Jerez de la Frontera, Spain
[21]Unit of Infectious Diseases and Clinical Microbiology, Hospital Universitario de Jerez de la Frontera, Jerez de la Frontera, Spain
[22]Department of Paediatrics, Consorci Hospital General Universitari de Valencia, Valencia, Spain
[23]Microbiology Department, Hospital La Fe, Valencia, Spain
[24]Paediatric Infectious Diseases and Immunodeficiencies Unit, Hospital Vall d'Hebron, Barcelona, Spain
[25]Unitat de Micobactèries, Laboratori Supranacional de Referència de l'OMS per a la Tuberculosis, Hospital Vall d'Hebron, Barcelona, Spain
[26]PROSICS, Institut Català de la Salut, Barcelona, Spain
[27]Department of Infectious Diseases, Rheumatology and Immunodeficiency, Hospital Universitario Virgen del Rocío, Sevilla, Spain
[28]Instituto de Investigación Biomédica de Sevilla (IBiS), Sevilla, Spain
[29]Clinical Microbiology, Infectious Diseases and Preventive Medicine Department, Hospital Universitario Virgen del Rocío, Sevilla, Spain
[30]Department of Paediatrics, Faculty of Medicine, The University of Melbourne, Parkville, Victoria, Australia
[31]Department of Infection, Immunity and Inflammation, Great Ormond Street Institute of Child Health, University College London, London, UK
Twitter David Aguilera-Alonso @DavidAguileraAl and Antonio Soriano-Arandes @tonisoriano66
Contributors BS-G and MT conceived of the study. BS-G, MT, DA-A, and JS-L designed the study. GS-G, DA-A and BS-G cleaned and analysed the data, and constructed the figures and tables. DA-A, GS-G, BS-G, JS-L and MT wrote the first draft of the manuscript. BS-G and DA-A are responsible for the overall content as guarantors. All authors contributed data to the study, contributed to the data interpretation, critically reviewed the manuscript and approved the final manuscript for submission. MT and BS-G are joint last authors.
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ORCID iDs
David Aguilera-Alonso http://orcid.org/0000-0003-1017-2386
Gonzalo Solís-García http://orcid.org/0000-0003-2256-8572
Antoni Noguera-Julian http://orcid.org/0000-0001-7485-0583
Julián González-Martín http://orcid.org/0000-0001-7248-6706
Fernando Baquero-Artigao http://orcid.org/0000-0002-6903-3826
Paula Rodríguez-Molino http://orcid.org/0000-0002-2259-0393
Iván Bloise Sánchez http://orcid.org/0000-0001-8392-0571
Jesús Saavedra-Lozano http://orcid.org/0000-0001-5971-011X
Daniel Blázquez-Gamero http://orcid.org/0000-0001-6483-8329
Paula López-Roa http://orcid.org/0000-0002-7989-4275
David Gomez-Pastrana http://orcid.org/0000-0002-6326-4076
Eva María López Medina http://orcid.org/0000-0003-2887-7181
Ana Gil-Brusola http://orcid.org/0000-0002-4418-3676
Antonio Soriano-Arandes http://orcid.org/0000-0001-9613-7228
Teresa Tórtola http://orcid.org/0000-0002-9188-298X
Lola Falcon-Neyra http://orcid.org/0000-0002-5808-8071
Verónica González Galán http://orcid.org/0000-0002-9302-7813
Marc Tebruegge http://orcid.org/0000-0003-4007-0807
Begoña Santiago-García http://orcid.org/0000-0002-4125-0423

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Alterações longitudinais na expetoração e nos mediadores inflamatórios no sangue durante os testes de supressão de FeNO

Simon Couillard,[1,2] Rahul Shrimanker,[1] Samuel Lemaire-Paquette,[2] Gareth M Hynes,[1] Catherine Borg,[1] Clare Connolly,[1] Samantha Jane Thulborn,[1] Angela Moran,[1] Sarah Poole,[1] Sophie Morgan,[1] Timothy Powell,[1] Ian Pavord,[1] Timothy Hinks[1]

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[1]Respiratory Medicine Unit, Nuffield Department of Medicine, University of Oxford, Oxford, UK
[2]Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, Sherbrooke, Quebec, Canada

Correspondence to
Dr Simon Couillard, Respiratory Medicine Unit, Nuffield Department of Medicine, University of Oxford, Oxford OX1 2JD, Oxfordshire, UK; s.couillard@usherbrooke.ca

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RESUMO

De modo a explorar se a não supressão de óxido nítrico exalado fracionado (FeNO) identifica a resistência aos corticosteroides, analisámos as alterações nos mediadores inflamatórios durante um teste de supressão de FeNO com terapia de corticosteroides de alta intensidade monitorizada. Em modelos lineares de efeitos mistos analisados ao longo do tempo, os 15 'supressores' clinicamente distintos (i.e., ≥42% supressão de FeNO) normalizaram as pontuações do Questionário de Controlo da Asma (média±DP, início ao fim do teste: 2,8±1,4 a 1,4±0,9, p<0. 0001) e as contagens de eosinófilos na expetoração (mediana (IQR), do início ao fim do teste: 29% (6%-41%) a 1% (1%-5%), p=0,0003) ao mesmo tempo que diminuíram significativamente os níveis de prostaglandina D_2 na expetoração (254 (89-894) para 93 (49-209) pg/ml, p=0,004) e diminuíram numericamente outros níveis de citocinas de tipo 2, quimiocinas e alarminas. Comparativamente, os 19 não supressores apresentaram eosinofilia persistente na expetoração (10% (1%-67%) apesar da terapia de alta intensidade) com níveis elevados de mediadores inflamatórios no final do teste (1,9 (0,9-2,8) vezes superior aos supressores). A não supressão de FeNO durante o tratamento monitorizado implica uma resistência biológica aos corticosteroides.

INTRODUCTION

Severe asthma represents 1 in 20 asthma cases but comprises half of asthma-related expenditure.[1] The biomarkers fractional exhaled nitric oxide (FeNO) and blood eosinophils are used in the clinic to identify higher risk type 2 inflammatory phenotype which responds favourably to anti-inflammatory therapy.[2 3]

The observation that FeNO predicts inhaled corticosteroid (ICS)-responsiveness has led to the development of the FeNO suppression test to identify non-adherence in difficult-to-treat, FeNO-high asthma.[4 5] One-third of patients have a persistently raised FeNO and disease burden despite objectively measured adherence to high-dose ICS.[4 6–8] This group of 'FeNO non-suppressors' have been presumed 'corticosteroid resistant',[9] but the longitudinal investigation of inflammatory changes over the course of a FeNO suppression test has not been reported. To explore the hypothesis that FeNO non-suppression identifies biological corticosteroid resistance, we analysed induced sputum and blood inflammatory mediator changes during a FeNO suppression test in patients who did and did not suppress FeNO.

METHODS

We performed an observational longitudinal analysis of FeNO suppression tests conducted in our specialist asthma clinic (Oxford, UK).

Patients ≥18 years old with asthma receiving high dosage ICS plus ≥1 other controller were recruited after multidisciplinary evaluation when they had persistently high FeNO (>40 ppb twice)[10] with no confounding pulmonary disease. Participants consented and underwent testing between January 2015 and February 2020; sputum induction and recruitment stopped in March 2020 due to the pandemic.

FeNO suppression tests were conducted according to an adaptation of an early protocol (see Figure E2, online supplement).[4] Briefly, patients with asthma underwent 7–35 days of additional inhaled and/or systemic corticosteroids (+1000 µg inhaled fluticasone propionate per day and, if FeNO did not suppress on day 7 according to the equation below, +80 mg intramuscular (IM) triamcinolone with follow-up 28 days later). Treatment adherence was monitored via a chipped inhaler (INCA) and/or nurse-administered triamcinolone injection. In addition to detailed clinical assessment, Asthma Control Questionnaire (ACQ)-5, spirometry, FeNO measurement (FeNO NIOX VERO), phlebotomy and sputum induction by hypertonic saline nebulisation in clinic on days 0, 7 and/or 35, patients performed daily FeNO measurements at home for days 1–6. Some FeNO suppression tests stopped after 7 days due to patient availability, physician decision or transition to research bronchoscopy protocols.

A positive FeNO suppression test was defined as a $Log_{10}\Delta FeNO \geq 0.24$, where $Log_{10}\Delta FeNO$ is calculated as: (mean (log_{10}FeNO day 0, log_{10}FeNO day 1)) − (log_{10}FeNO day 35 or, if unavailable, mean (log_{10}FeNO day 6, Log_{10}FeNO day 7)). Conversely, patients with a negative FeNO suppression test (ie, <42% fall in FeNO) were categorised as 'non-suppressors'.[4] Medical notes and forms completed on day 0, 7 and 35 were reviewed to assess whether evidence of pre-existing nonadherence issues had been documented. Triggers for categorising patients as 'previously non-adherent' were any of: (1) adequate chipped inhaler data showing <70% acceptable doses taken during the first 7 days of the test, (2) 'non-adherent' noted during clinical review by specialist nurse or (3) nursing note stating significant inhaler technique difficulties persisting throughout the test.

Longitudinal (days 0, 7 and 35 whenever available) samples were analysed for 28 clinical, biomarker, sputum and serum inflammatory mediators. Inflammatory proteins were measured in duplicates using multiplex electrochemiluminescent assays (Meso Scale Discovery, USA) or single ELISA (Cayman Chemical, USA).

Demographics for FeNO suppressors versus non-suppressors were compared by unpaired t-tests for parametric variables, Mann-Whitney tests for nonparametric variables, and Fisher's exact test or χ^2 for categorical variables. To test our hypothesis that FeNO non-suppressors exhibit biological resistance, longitudinal analyses were

Table 1 Baseline subject characteristics

Parameter	FeNO suppressed n=15	Not suppressed n=19	P value
Age, years	42±13	57±16	0.006
Male	5 (33)	10 (53)	ns
BMI, kg/m^2	26±4	28±5	ns
Comorbidities			
Atopy*	12 (80)	12 (63)	ns
Nasal polyps	7 (47)	7 (37)	ns
Gastro-oesophageal reflux	2 (13)	3 (16)	ns
Cardiovascular disease	2 (13)	1 (5)	ns
Smoking status: never-smoker	12 (80)	11 (58)	ns
Ex-smoker	2 (13)	7 (37)	
Current smoker	1 (7)	1 (5)	
ACQ-5 score at baseline	2.8±1.4	2.5±1.5	ns
Asthma attacks in past year†	1 [0–3]	4 [0–5]	ns
ICS, BDP-CFC eq., µg/day	1561±502	1921±344	0.02
On maintenance OCS	3 (20)	9 (47)	ns
FEV_1, % predicted	89±19	78±17	ns
FEV_1/FVC ratio, % observed	75±17	67±11	ns
FeNO ppb	119 [75-190]	94 [60-136]	ns
Blood eosinophils, cells×10^9/L	0.54 [0.50–0.83]	0.46 [0.36–0.59]	0.03
Total IgE levels, kU/L	545 [35–1551]	229 [77-359]	ns
Sputum eosinophils, %	29 [7-41]	13 [3-39]	ns
Sputum neutrophils, %	46 [19-61]	68 [32-77]	ns
Inadequate adherence identified	8 (53)	2 (11)	0.007
Test duration			
7 days	5 (33)	11 (58)	ns
35 days	10 (67)	8 (42)	
Test optimisation method:			
+FP 1000 µg inhaled-only	12 (80)	13 (68)	ns
+FP then Triamcinolone 80 mg IM	3 (20)	6 (32)	
No of samples (days 0, 7, 35)			
Sputum differential cell count	21 {7, 10, 4}	17 {8, 7, 2}	
Sputum supernatant	25 {9, 9, 7}	31 {13, 12, 6}	
Serum	30 {11, 10, 9}	41 {17, 16, 8}	

Data are presented as no (%), mean±SD, median (IQR), or total no of samples (days 0, 7, 35).
P values reported are unpaired t-tests for parametric variables, Mann-Whitney U tests for nonparametric variables, Fisher's exact test or χ^2 for categorical variables.
*Atopy defined as patient-reported allergic rhinitis, eczema, allergen-worsening of asthma or food allergy.
†Asthma attacks are defined as acute asthma episodes requiring 3 days or more of systemic corticosteroids.
ACQ-5, Asthma Control Questionnaire-5 Item; BDP-CFC eq., beclomethasone dipropionate with CFC propellant equivalent; BMI, body mass index; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s (postbronchodilator); FP, fluticasone propionate; FVC, forced vital capacity; ICS, inhaled corticosteroid; IM, intramuscular; ns, not significant; OCS, oral corticosteroids.

performed for the 28 outcome repeated measures (days 0, 7 and 35 whenever data were available; plus home-FeNO measurement on days 1–6) in linear mixed-effects models with a random intercept on same patients for (1) FeNO non-suppressors alone and FeNO suppressors alone, respectively, assessing significance of change over timepoints in each subgroup; and (2) FeNO suppressors versus FeNO non-suppressors, assessing significance of the group × time interaction (ie, whether change over time was different according to group status). Significant findings in the longitudinal groupwise analyses were further explored in pooled linear mixed effects models assessing the relationship between selected continuous outcomes (ie, the dependent variable; log-transformed when required) and FeNO (independent variable; log-transformed). Modelling assumptions were all verified visually with appropriate diagnostic plots. The primary set of linear mixed-effect models' p values (84 models) were controlled for a false discovery rate <0.05 using the Benjamini-Hochberg procedure[11]; other statistics used a two-sided α=0.05. Linear mixed-effects models were computed in RStudio 2021.09.01 build 372 (RStudio, USA) with R V.4.1.2 (R Foundation), and other statistics were performed in SPSS V.28 (IBM) and GraphPad Prism V.9.3.1 (GraphPad, USA).

RESULTS

Eighty-seven patients were referred for FeNO suppression testing between January 2015 and February 2020; 34 completed tests were included (see online supplemental appendix 1). There were two protocol deviations when FeNO non-suppressors were not administered IM triamcinolone on day 7 due to incorrect application of the FeNO suppression equation stated in the study methods (eg, using only 1 day to determine if suppressed, rather than the mean of several days).

Nineteen patients did not suppress FeNO: these were significantly older, on higher background ICS dosage, had lower baseline blood eosinophil count and had little or no adherence/inhaler technique issues noted (table 1). Specimen availability was low, especially for sputum differential cell counts, but there was no difference in the number of sputum inductions achieved between groups and no trend for better/worst sampling success according to study day.

The clinical, biomarker and sputum/serum inflammatory longitudinal responses during the FeNO suppression tests are shown in table 2, and linear mixed-effect models' outputs are detailed in online supplemental appendix 2. In FeNO suppressors alone, ACQ-5 scores improved significantly during the test (days 0, 7, 35; mean±SD: 2.8±1.4, 1.6±0.9, 1.3±1.0, p<0.0001 over time), as did sputum eosinophils (median (IQR): 29 (6–41), 3 (1–11), 2 (1–5) %, p=0.0003) and sputum PGD_2 (254 (89–894), 174 (37–341), 93 (53–196) pg/mL, p=0.004). In FeNO non-suppressors alone, only the longitudinal change in sputum IL-4 (1.0 (0.3–1.1), 1.0 (0.5–1.2), 0.1 (0.1–0.3) pg/mL, p=0.004) was retained after correcting for multiplicity of testing. When comparing FeNO suppressors and non-suppressors, only the longitudinal change in FeNO was significantly different after correcting for multiplicity of testing (↓3.4 (2.3–4.2) vs ↓1.5 (1.1–1.7)-fold, p<0.0001 for group × time interaction).

The results of the above subgroup longitudinal analyses were further explored for ACQ-5, sputum eosinophils, sputum PGD_2 and sputum IL-4. The continuous relationship between FeNO suppression and these analytes are detailed in online supplemental appendix 3. In effect, a 42% decrease in FeNO is associated with a significant change in ACQ-5 (↓0.31 (95% CIs: 0.20 to 0.42) points, p<0.0001), sputum eosinophils (↓ 1.37 (1.10 to 1.72)-fold, p=0.009) and sputum PGD_2 (↓ 1.16 (1.01 to 1.32)-fold, p=0.04). There was no significant relationship between the degree of FeNO suppression and sputum IL-4.

The four analytes found to significantly change in both subgroup and pooled continuous analyses according to FeNO suppression (FeNO, ACQ-5, sputum eosinophils and PGD_2) are plotted (figure 1).

It is noteworthy that more outcome measures decreased with p<0.05 in FeNO suppressors than non-suppressors (11/28 vs 6/28, p=0.14 on χ^2 test), and in nearly all cases the end-test median values for sputum and serum inflammatory mediators were numerically greater in FeNO non-suppressors than suppressors (1.9 (0.9–2.8)-fold; 15/22 values greater in FeNO non-suppressors, p=0.02 on χ^2 test). Patients who did not suppress FeNO also had significantly greater FeNO values at test termination than suppressors (56 (43–123) vs 35 (20–55) ppb, unpaired t-test on log-FeNO values p=0.001). These trends were especially striking for sputum

Table 2 Before-and-after clinical and inflammatory changes according to FeNO suppression test result

Analyte (pg/mL or stated) LLOD*		FeNO suppressed			FeNO not suppressed			
		Before	After	P for time (n analysed)	Before	After	*P* for time (n analysed)	P for group ×time
Clinical	**ACQ-5 score**	2.8±1.4	1.4±0.9	**<0.0001** (n=15)	2.5±1.5	1.9±1.3	ns (n=19)	ns
	FEV_1 (L)	2.79±0.86	3.05±0.96	0.009 (n=15)	2.39±0.92	2.56±0.89	0.04 (n=19)	ns
	FEV_1 (% pred)	89±19	98±19	0.02 (n=15)	78±17	83±18	ns (n=19)	ns
	FEV_1/FVC (%)	75±17	78±12	ns (n=15)	67±11	70±10	ns (n=19)	ns
Biomarker	**FeNO (ppb)**	119 [75–190]	35 [20–55]	**<0.0001** (n=15)	94 [60–136]	56 [43–123]	**<0.0001** (n=19)	**<0.0001**
	Blood Eos (×109/L)	0.54 [0.50–0.83]	0.42 [0.10–0.60]	0.02 (n=15)	0.46 [0.26–0.58]	0.24 [0.19–0.36]	ns (n=19)	ns
Induced sputum mediators	**Eosinophils (%)**	29.3 [6.5–41.3]	1.3 [1.0–5.3]	**0.0003** (n=11)	13.0 [2.9–38.8]	10.0 [1.1–67.0]	ns (n=10)	ns
	Neutrophils (%)	46.3 [9.8–61.3]	16.0 [4.7–74.7]	ns (n=11)	67.8 [32.0–77.3]	40.3 [8.5–70.3]	ns (n=10)	ns
	IL-4 0.2	0.4 [0.1–1.0]	0.1 [0.1–0.6]	ns (n=11)	1.0 [0.3–1.1]	0.5 [0.1–1.0]	**0.002** (n=13)	ns
	IL-5 0.5	3.7 [1.2–20.9]	1.4 [0.6–6.0]	0.045 (n=11)	7.8 [1.9–14.5]	3.9 [2.0–7.4]	ns (n=13)	ns
	IL-13 4.2	6.9 [5.7–15.8]	8.8 [6.0–15.5]	ns (n=11)	7.7 [5.4–10.5]	7.7 [6.3–10.8]	ns (n=13)	ns
	IL-33 0.6	1.4 [0.3–1.4]	0.3 [0.3–0.7]	0.02 (n=11)	1.6 [1.4–2.0]	1.4 [0.4–1.7]	0.02 (n=13)	ns
	TSLP 0.9	3.6 [1.3–13.9]	3.0 [1.1–7.9]	0.008 (n=11)	7.0 [5.0–13.4]	6.9 [4.1–10.3]	ns (n=13)	ns
	Eotaxin-3 4.2	63 [24–410]	58 [14–257]	ns (n=9)	361 [20–677]	169 [48–329]	ns (n=11)	ns
	TARC 0.4	10 [7–79]	16 [5–42]	ns (n=9)	36 [8–208]	31 [17–48]	ns (n=11)	ns
	LTE_4 7.8	305 [74–830]	106 [46–218]	0.01 (n=11)	226 [54–905]	80 [47–677]	ns (n=13)	ns
	PGD_2 19.5	254 [89–894]	93 [49–209]	**0.004** (n=11)	279 [151–366]	176 [119–320]	0.04 (n=13)	0.01
	IFN-γ 0.3	0.6 [0.2–1.7]	0.2 [0.2–0.3]	ns (n=9)	0.2 [0.2–0.4]	0.4 [0.2–1.1]	ns (n=11)	ns
	TNF 0.4	1.8 [0.2–9.8]	0.5 [0.2–2.4]	ns (n=9)	1.7 [0.9–4.0]	1.7 [0.2–7.3]	ns (n=11)	ns
Serum mediators	**IL-4** 0.1	0.1 [0.1–0.1]	0.1 [0.1–0.1]	ns (n=14)	0.1 [0.1–0.1]	0.1 [0.1–0.1]	ns (n=19)	ns
	IL-5 0.4	1.4 [0.6–3.4]	0.8 [0.5–1.2]	ns (n=14)	1.5 [0.4–2.4]	0.6 [0.5–1.6]	ns (n=19)	ns
	IL-13 6.7	9.5 [3.3–12.0]	3.3 [3.3–8.5]	ns (n=14)	3.3 [3.3–12.8]	6.1 [3.3–10.5]	ns (n=19)	ns
	IL-33 0.4	0.8 [0.2–0.8]	0.2 [0.2–0.8]	ns (n=14)	0.6 [0.2–0.8]	0.4 [0.2–0.8]	ns (n=19)	ns
	TSLP 0.5	1.8 [0.8–3.1]	1.8 [1.1–2.5]	ns (n=14)	2.7 [1.8–3.6]	2.4 [1.6–3.8]	ns (n=19)	ns
	Eotaxin-3 4.2	14 [6–30]	15 [10–30]	ns (n=14)	19 [10–35]	17 [9–32]	0.03 (n=19)	ns
	TARC 0.2	281 [167–561]	318 [160–560]	0.01 (n=14)	247 [144–395]	248 [92–406]	ns (n=19)	ns
	IFN-γ 0.3	0.6 [0.2–1.1]	0.4 [0.3–0.7]	ns (n=14)	0.3 [0.2–1.0]	0.3 [0.2–0.8]	ns (n=19)	ns
	TNF 0.4	0.8 [0.2–1.3]	1.0 [0.5–1.9]	ns (n=14)	1.1 [0.2–2.1]	0.9 [0.2–2.0]	ns (n=19)	ns

Data are presented as mean±SD or median (IQR); units of measured are in pg/mL unless otherwise stated.
Bold p-values are those retained after controlling for multiplicity of testing (false discovery threshold 0.05 across 84 analyses). P values reported were obtained by linear mixed effects models.
*Cytokine levels that were not quantified were assigned the arbitrary value of 0.5×the LLOD (value below the row label when appropriate) to allow analysis.
ACQ-5, 5-item Asthma Control Questionnaire; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s s (postbronchodilator); FVC, forced vital capacity; IFN, interferon; IL, interleukin; LLOD, lower limit of detection; LTE4, leukotriene E4; ns, not significant; PGD2, prostaglandin D2; TARC, thymus activation regulated cytokine (CCL17); TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin.

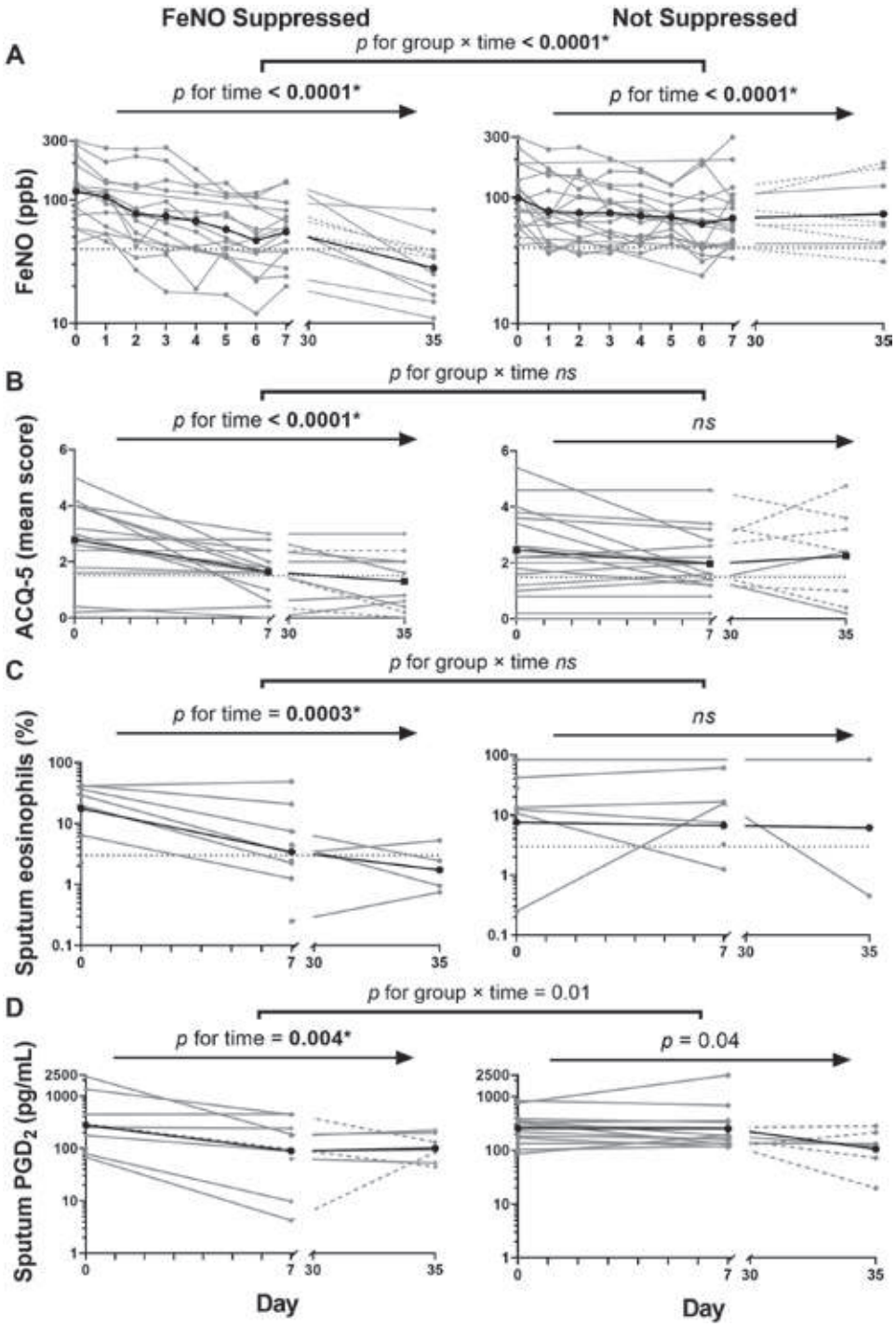

Figure 1 Longitudinal changes in selected analytes during a fractional exhaled nitric oxide (FeNO) suppression test stratified by its results. (A) FeNO (individual and geometric mean values), (B): 5-item Asthma Control Questionnaire (ACQ-5) (individual and mean values); (C): sputum eosinophils (individual and geometric mean values); (D): sputum prostaglandin D_2 (PGD_2) (individual and geometric mean values). Bold *p values are those retained after controlling for a false discovery rate <0.05; dashed segments (---) indicate patients administered IM triamcinolone on day 7; dotted horizontal lines (···) delineate the limits of normal/controlled asthma for FeNO (<40 ppb), ACQ-5 (<1.5) and sputum eosinophils (<3%).[10] ns, not significant.

eosinophils (figure 1C), which decreased 5.4 (2.4–10.3)-fold in FeNO suppressors (~normal median end-test value: 1 (1–5) %, n=7) while increasing 1.3 (0.6–1.6)-fold in non-suppressors (~high median end-test value: 10 (1–67) %, n=6).

Finally, sensitivity analyses were conducted to assess whether the final degree of FeNO suppression or ACQ-5 improvement varied according to study duration (7 days or 35 days) and the optimisation method (ICS-only or ICS+IM triamcinolone) (online supplemental appendix 4). The results suggest that, although methods to ensure optimal FeNO suppression varied, the magnitude of change did not differ significantly between study durations and interventions.

DISCUSSION

We found that patients who failed to suppress FeNO after a suppression test had no improvement in symptoms and FeNO, reflected by raised sputum eosinophil counts, sputum PGD_2, and other inflammatory protein levels at the end of the test. In contrast, FeNO suppressors improve significantly in these domains, often reaching normal values. These results suggest that the assessment of biological corticosteroid resistance can be based on a failure to suppress FeNO during monitored high-intensity corticosteroid therapy.

The criterion for FeNO suppression was derived to identify pre-existing nonadherence—not to assess corticosteroid-resistant

type-2 inflammation.[4] Nevertheless, patients who failed to suppress FeNO have consistently been found to be older males with higher baseline asthma morbidity and lesser longitudinal improvements in symptom scores, lung function, and FeNO.[4 6–8] Our data confirm these distinct clinical characteristics and provide translational data supporting the concept that FeNO non-suppression identifies corticosteroid resistance.[9] They also highlight how monitoring adherence allows better interpretation of FeNO fluctuations.[12] An important strength of our study is that we rigorously controlled for multiplicity of testing. Furthermore, we validated the significant findings from longitudinal subgroup analyses (FeNO suppressed, not suppressed) by modelling them according to the degree suppression of FeNO. Hence, FeNO suppression (taken both as a categorical and a continuous variable) translates to a normalisation of the ACQ-5 score, sputum eosinophil count and sputum PGD_2; a mast cell-produced eicosanoid with proinflammatory and bronchoconstrictive effects.[13] Conversely, the clinically distinct FeNO non-suppressors have corticosteroid-refractory symptoms and airway inflammation.

Notwithstanding the results of our subgroup longitudinal analy-ses which confirmed our study hypothesis, we were unable to show a comparative difference between the two groups across time, possibly because the assessment of the group×time statistical interaction was underpowered to detect the likely difference. Sputum availability in our cohort was also problematic and the study was thus generally underpowered despite robust linear mixed-effect modelling efforts to use all the data at hand. Reports on sputum induction success rates reach 92%[14]; our rate was 44% for differential cell counts and 65% for sputum supernatants. Serum samples were more available (83%) but less useful to assess FeNO-related mechanisms. Another limitation of this study is its observational design with consequent heterogenous testing durations and interventions, although sensitivity analyses did not show any significant impact of these factors on FeNO and symptom improvements. Despite these limitations, the number of inflammatory mediator changes in contrasting directions between suppressors and non-suppressors were unlikely to be just stochastic.

To conclude, our longitudinal subgroup support the notion that patients with uncontrolled asthma who fail to suppress FeNO despite monitored high-intensity corticosteroid therapy have distinct clinical, biomarker and inflammatory mediator responses which imply biological corticosteroid resistance. Further comparative biological analyses between FENO suppressors and non-suppressors require larger validation cohorts and sample sets.

Twitter Simon Couillard @simcouillard and Timothy Hinks @HinksLab
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Competing interests SC and TP: received a non-restricted research grant from Sanofi-Genzyme for investigator-initiated type 2 innovation research, within the submitted work; SC: received speaker honoraria from GlaxoSmithKline, Sanofi-Regeneron and AstraZeneca, outside the submitted work. RS has no conflicts to declare. SL-P has no conflicts of interest to declare. GMH reports funding from an Oxford-UCB Prize Fellowship and the NIHR BRC, outside the submitted work. CB, CC, SM, AM, SP and SJT have no conflicts to declare. TP is an employee of Springer Nature, outside the submitted work. IDP: In the last 5 years, IP has received speaker's honoraria for speaking at sponsored meetings from AstraZeneca, Boehringer Ingelheim, Aerocrine AB, Almirall, Novartis, Teva, Chiesi, Sanofi/Regeneron, Menarini, and GSK, and payments for organising educational events from AstraZeneca, GSK, Sanofi/Regeneron, and Teva. He has received honoraria for attending advisory panels with Genentech, Sanofi/Regeneron, AstraZeneca, Boehringer Ingelheim, GSK, Novartis, Teva, Merck, Circassia, Chiesi, and Knopp, and payments to support FDA approval meetings from GSK. He has received sponsorship to attend international scientific meetings from Boehringer Ingelheim, GSK, AstraZeneca, Teva, and Chiesi. He has received a grant from Chiesi to support a phase 2 clinical trial in Oxford. He is co-patent holder of the rights to the Leicester Cough Questionnaire and has received payments for its use in clinical trials from Merck, Bayer, and Insmed. In 2014-2015 he was an expert witness for a patent dispute involving AstraZeneca and Teva. TH: has received grants from the Wellcome Trust, grants from The Guardians of the Beit Fellowship, grants from Pfizer Inc., Kymab Ltd, grants from University of Oxford, and grants from the NIHR Oxford BRC outside the submitted work. He has received personal fees from Astra Zeneca, personal fees from TEVA, personal fees from Omniprex and personal fees from Peer Voice outside the submitted work.
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ORCID iDs
Simon Couillard http://orcid.org/0000-0002-4057-6886
Rahul Shrimanker http://orcid.org/0000-0002-2730-9978
Gareth M Hynes http://orcid.org/0000-0002-9317-2711
Clare Connolly http://orcid.org/0000-0003-2943-7911
Samantha Jane Thulborn http://orcid.org/0000-0002-9379-6655
Angela Moran http://orcid.org/0000-0002-2067-9335
Sophie Morgan http://orcid.org/0000-0003-0298-7614
Timothy Powell http://orcid.org/0000-0002-9208-674X
Ian Pavord http://orcid.org/0000-0002-4288-5973
Timothy Hinks http://orcid.org/0000-0003-0699-2373

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Indústria tabaqueira detentora de empresas farmacêuticas: um inquérito internacional a pessoas com doenças respiratórias

Ruth Tal-Singer,[1,2] Linda Walsh,[1] Tim Deuby,[1] Angela Shaughnessy,[1] Karin F Hoth,[3] Victor Gascon,[2] Susanne Hintringer,[2] Nicole Hass,[2,4] Tonya Winders,[2] Lucy Morgan,[5] Byron Thomashow,[1] David M Mannino[1]

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[1]COPD Foundation, Miami, Florida, USA
[2]Global Allergy and Airways Patient Platform (GAAPP), Vienna, Austria
[3]Department of Psychiatry, university of Iowa, Iowa City, Iowa, USA
[4]Spanish COPD Association, Asturia, Spain
[5]Department of Respiratory Medicine, Concord Repatriation General Hospital, Sydney, New South Wales, Australia

Correspondence to
Dr Ruth Tal-Singer, COPD Foundation, Miami, FL 33134, USA;
rtalsinger@copdfoundation.org

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RESUMO

A aquisição da empresa farmacêutica respiratória Vectura pela Phillip Morris International em 2021 foi criticada pela comunidade médica e de saúde pública, enquanto conflito de interesses, com pouca informação até à data, por parte da comunidade de doentes ou do público. Para colmatar esta lacuna, a Fundação COPD, juntamente com parceiros globais, realizou um inquérito a 1196 pessoas com doenças respiratórias crónicas. 70% sentiram-se incomodadas por uma empresa tabaqueira produzir um inalador para tratar doenças pulmonares e 48% relataram que iriam querer trocar os inaladores se soubessem que uma empresa tabaqueira produzia ou vendia os seus inaladores. Os doentes preocupam-se com quem faz as terapias utilizadas para tratar as suas doenças.

INTRODUCTION

In 2021, the tobacco company Phillip Morris International (PMI) purchased the UK-based pharmaceutical company Vectura.[1] Based on its global market share of 12%, PMI tobacco sales may well be contributing to more than a million deaths annually, with many of those deaths from chronic obstructive pulmonary disease (COPD).[2] Vectura has been part of the development of several widely used medication delivery devices that treat chronic respiratory diseases, including COPD and asthma.[2] The purchase of Vectura by PMI has had repercussions in the medical community where, for example, medical societies prohibit the participation of the tobacco industry in conferences and publications, based on their prior history of misrepresenting the impact of smoking, spreading disinformation and other misbehaviours.[3] This expansion of the tobacco industry into pharmaceutical companies follows their recent entry into non combusted tobacco products; all major international tobacco companies now have a presence in e-cigarettes.[4]

A group that has not been heard from, to date, is the patient community of people with COPD and other respiratory diseases, many of whom have tobacco-related disease. To address this gap, the COPD Foundation, supported by its partners the Global Allergy and Airways Patient Platform and the Lung Foundation of Australia, distributed a patient survey inquiring about patients' attitudes regarding a tobacco company owning the company that makes their respiratory inhaler devices with the aim to inform global advocacy efforts.

METHODS

This cross-sectional survey was developed by the COPD foundation with input from patient advocates. It was translated to Spanish and German by members of the Global Allergy and Airways Patient Platform. Respondents were eligible if they were adults above 18 years old. Analyses were limited to current or former uses of respiratory inhalers to treat chronic lung conditions, irrespective of their smoking status. The survey was admi-nistered anonymously between January 2022 and March 2022 and advertised using various social media and patient advocacy community support platforms; posts in English used an explainer video on the rationale for the survey and recruiting inhaler users. The survey consisted of 13 questions exploring respondent demographics, comorbidities and attitudes towards pharmaceutical industry acquisition by tobacco companies. All responses remained strictly confidential.

The survey was administered in three languages (English, Spanish and German) and reported using the Survey Monkey platform.

This report includes a composite of responses to the three survey versions analysed in Microsoft Excel. We graded responses to the question 'In your own words, what is your opinion of tobacco companies owning companies that are earning money from inhalers and/or medications for lung conditions?' as positive, neutral or negative; grading was reviewed by two raters. A third reviewer decided the grade when there was disagreement between the primary reviewers.

RESULTS

The complete survey was only available to people who reported inhaler use, reducing the number of respondents from 1628 in the original sample to 1196 in the analysed sample. Of the 1196, 1033 (86.4%) responded in English, 136 (11.4%) responded in Spanish and 27 (2.2%) responded in German (table 1, online supplemental tables 1, 2 and 3). Current inhaler use was reported by 94%, with an additional 6% reporting former inhaler use (table 2).

Among the respondents, most (68%) were from North America, although all regions of the world were represented (online supplemental figure 1). Thirty-four per cent were from cities with a population of 100 000 or higher. Most were former smokers (73%), with 11% reporting current smoking ad 15% never smoking. In contrast, current vaping was only reported by 2% with former vaping in an additional 12%. COPD was reported by 78% of respondents (table 1).

When asked 'If you knew that a tobacco company owned the company that makes or sells your inhaler, would you want to switch to a different inhaler?' 48% (n=571) responded 'Yes' and 17% (n=208) responded 'No', with the remainder either not sure (n=398, 33%) or not answering (n=19, 2%; figure 1A). This desire to switch was reported by 47% of 133 current smokers, 45% of 874 former smokers and 60% of 178 never smokers.

In response to the question 'How do you feel about a tobacco company making money from an inhaler that treats lung conditions because they

Table 1 Details of survey respondents

	English	Spanish	German	Total (per cent)
Total respondents	1033	136	27	1196
Have you ever been diagnosed with any of the conditions listed below? (multiple selections allowed)				
Chronic obstructive pulmonary disease	859	61	14	934 (78.1)
Emphysema	519	16	6	541 (45.2)
Bronchiectasis	287	32	9	328 (27.4)
Asthma	384	56	10	450 (37.6)
Alpha-1 antitrypsin deficiency	27	0	0	27 (2.3)
Bronchiectasis	97	10	3	110 (9.2)
Nontuberculous mycobacterial lung disease	19	5	0	24 (2.0)
I am not sure	5	6	0	11 (0.9)
I prefer not to answer	15	4	3	22 (1.8)
Other (ILD, EGPA, etc.)	44	12	2	58 (4.8)
Have you ever smoked tobacco products?				
Yes, I smoke now	103	21	9	133 (11.1)
Yes, but I quit smoking	789	72	13	874 (73.1)
No, I never Smoked	131	42	5	178 (14.9)
Missing	10	1	0	11 (0.9)
Have you ever vaped tobacco products?	26	0	3	29 (2.4)
Yes, I vape now	125	14	6	145 (12.1)
Yes, but I quit vaping	875	121	17	1013 (84.7)
No, I never vaped	7	1	1	9 (0.8)
I live in a:				
Large city (greater than 500 000 people)	202	63	5	270 (6)
Big city (between 100 000 and 500 000 people)	139	42	7	188 (15.7)
Medium-sized city (between 20 000 and 100 000 people)	279	19	5	303 (25.3)
Small town (between 2500 and 20 000 people)	244	9	3	256 (21.4)
Rural or remote area (under 2500 people)	147	1	7	155 (13.0)
I am not sure	11	2	0	13 (1.1)
I prefer not to answer	11	0	0	11 (0.9)

EGPA, eosinophilic granulomatosis with polyangiitis; ILD, interstitial lung disease.

own part of the company that makes them?' 43% responded 'It really bothers me' with an additional 27% responding 'It bothers me'. Only 19% were not bothered by this scenario (figure 1B).

For analysis of the question 'In your own words, what is your opinion of tobacco companies owning companies that are earning money from inhalers and/or medications for lung conditions?' the overall patient sentiment was negative. A total of 655 of the 844 (78%) free-text responses were negative ('greedy', 'disgusting', shameful—they should be shut down), while 90 (11%) were neutral ('I do not know', 'does not matter') and 99 (12%) were positive ('no problem if the inhaler works', 'good for them').

DISCUSSION

This survey reports patients' opinions on how they view tobacco industry ownership of companies that develop formulations or devices for inhaled therapies and sell therapies that are used to treat chronic respiratory disease. The majority (70%) of patients using inhalers in this survey were bothered by this scenario and 48% said they would want to change inhalers if they knew that a tobacco industry company was making or selling their inhaler.

Table 2 Inhaler use

	English	Spanish	German	Total (per cent)
Total respondents	1033	136	27	1196
Which of the following is most important to you				
When choosing your inhaler?				
My insurance will pay for it	206	9	8	223 (18.6)
The cost of the inhaler if I have to pay for it	107	10	0	117 (9.8)
It makes me feel better	394	37	8	439 (36.7)
It is easy to use	24	8	0	32 (2.7)
My doctor recommends it	256	67	9	332 (27.8)
I am not sure	13	3	2	18 (1.5)
I prefer not to answer	4	2	0	6 (0.5)
Other	5	6	0	11 (0.9)
Missing	29	0	0	29 (2.4)
Have you ever used an inhaler to treat your lung condition?				
Yes, I currently use my inhaler every day (maintenance treatment)	831	70	18	919 (76.8)
Yes, I currently use my inhaler only when I have symptoms (rescue inhaler)	161	37	4	202 (16.9)
I was prescribed an inhaler in the past, but I don't use it now	41	29	5	75 (6.3)
No, I have never used an inhaler*	0	0	0	0 (0.0)

*Survey was limited to respondents who reported former or current inhaler use.

In this survey, the most important factor to patients in choosing their inhaler was 'It makes me feel better' (table 2). Discussions between patients and providers should now include a frank discussion about involvement of the tobacco industry in these therapies as this may also affect what therapies a patient may want to be on.[5]

There are several limitations of this study. Participants were volunteers recruited via the outreach efforts of the study sponsors and may not reflect the views of the broader population of patients with lung disease. Since patient use of medications, including inhalers, is influenced by several factors, including insurance coverage, structure of the healthcare system, ability to properly use the device, convenience and other factors,[6–9] the willingness to switch medications that was expressed in this survey may not actually occur.

To conclude, these results highlight that patients are interested in how their medications are developed and who profits from their

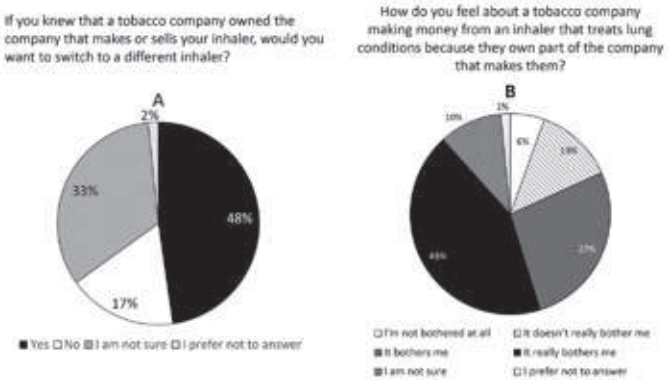

Figure 1 (A) Responses to 'if you knew that a tobacco company owned the company that makes or sells your inhaler, would you want to switch to a different inhaler'; (B) Responses to 'How do you feel about a tobacco company making money from an inhaler that treats lung conditions because they own part of the company that makes them' (n=1196 survey participants provided a response; integrated dataset English Spanish and English surveys).

sales and that the concern expressed by professional societies and the medical community is reflected in the patient community.

Twitter Ruth Tal-Singer @rtalsinger1 and Victor Gascon @MachinaVictor

Contributors RT-S, TD, LW, DMM, KFH, BT and AS designed the survey. VG and SH translated the survey and TW, NH and LM supported dissemination within patient community networks. RT-S, TD and AS analysed the survey. DMM and RT-S drafted the manuscripts and all authors reviewed and commented on the final version. Survey was funded by the COPD foundation.

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Competing interests RT-S is a retiree and shareholder of GlaxoSmithKline. She is a non-executive board member of ENA Respiratory and reports holding share options. She also reports personal fees from Vocalis Health, ENA Respiratory, Teva, and Immunomet. TW is President & CEO of Allergy & Asthma Network in the USA. She serves as President of GAAPP. Both AAN and GAAPP receive funding for unbranded disease awareness, education and advocacy from Aimmune, ALK, Amgen, AZ, DBV, GSK, Novartis, Sanofi/Regeneron, Viatris, TEVA. TW personally reports speaker & advisor fees from Aimmune, ALK, Amgen, AZ, DBV, Novartis and Sanofi/Regeneron. BT reports consultancy to GlaxosmithKline and participation at an advisory board for Boehringer Ingelheim. DMM is a former employee and current shareholder of GlaxoSmithKline and receives royalties from Up to Date. He is also a consultant to GlaxoSmithKline, Medical Director of the COPD Foundation, and an expert witness for people suing the Tobacco Industry. LW, TD, AS, KFH, VG, SH, NH and LM report no conflicts.

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CURSO DE ESPECIALIZAÇÃO

DOENÇAS RESPIRATÓRIAS E DO SONO DA CRIANÇA

CURSOS AVANÇADOS EM REGIME B-LEARNING

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