

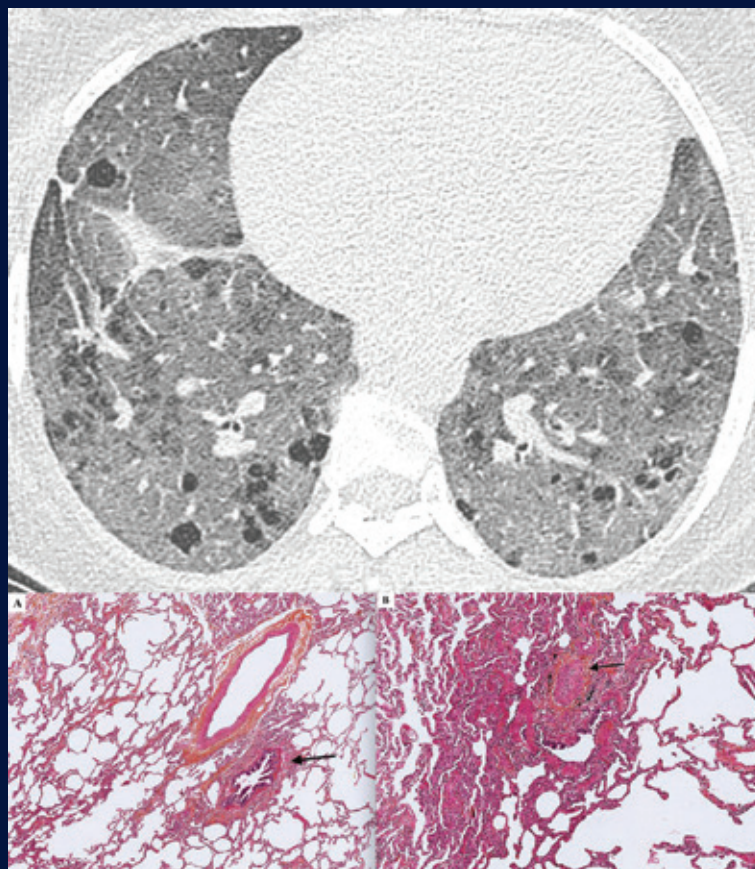
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O perfil de segurança de Revinty foi validado em ensaios clínicos de grande escala, como o SUMMIT⁴ (n=4121) e SLS na Asma (n=2114)¹

1) Woodcock A *et al.* Lancet 2017; 390:2247–2255. 2) Bateman ED *et al.* Thorax 2014; 69:312–319. 3) GINA. Global strategy for asthma management and prevention, 2021. Available at: <https://ginasthma.org/gina-reports/> (Acedido: dezembro 2021). 4) Vestbo J *et al.* Lancet 2016;387:1817-1826. ICS: Corticosteroide inalado; LABA: Agonista β_2 de longa duração de ação.

INFORMAÇÕES ESSENCIAIS COMPATÍVEIS COM O RCM. NOME DO MEDICAMENTO Revinty Ellipta **COMPOSIÇÃO QUALITATIVA E QUANTITATIVA** Revinty Ellipta 92/22 mcg: Cada inalação disponibiliza uma dose administrada de 92 mcg de furoato de fluticasona e 22 mcg de vilanterol (como trifrenatato). Isto corresponde a um recipiente unidose de 100 mcg de furoato de fluticasona e 25 mcg de vilanterol (como trifrenatato). Revinty Ellipta 184/22 mcg: Cada inalação disponibiliza uma dose administrada de 184 mcg de furoato de fluticasona e 22 mcg de vilanterol (como trifrenatato). Isto corresponde a um recipiente unidose de 200 mcg de furoato de fluticasona e 25 mcg de vilanterol (como trifrenatato). Cada dose administrada contém aproximadamente 25 mg de lactose mono-hidratada **FORMA FARMACÉUTICA** Pó para inalação em recipiente unidose **INDICAÇÕES TERAPÉUTICAS Asma:** Revinty Ellipta 92/22 mcg e 184/22 mcg está indicado para o tratamento regular da asma em adultos e adolescentes com idade ≥ 12 anos em que a utilização de um medicamento contendo uma associação (agonista β_2 de ação prolongada e corticosteroides para inalação) é adequada: doentes que não estão adequadamente controlados com corticosteroides para inalação e com agonistas β_2 , de curta duração de ação 'conforme o necessário'; doentes que estão já adequadamente controlados com corticosteroides para inalação e com agonistas β_2 , de ação prolongada. **DPOC:** Revinty Ellipta 92/22 mcg está indicado para o tratamento sintomático de adultos com DPOC com um FEV₁ previsível normal $<70\%$ (após o broncodilatador) com antecedentes de exacerbação apesar da terapêutica regular com um broncodilatador. **POSOLOGIA E MODO DE ADMINISTRAÇÃO Asma (92/22 mcg e 184/22 mcg)** **Adultos e adolescentes ≥ 12 anos** Deve considerar-se uma dose inicial de uma inalação de 92/22 mcg uma vez por dia para adultos e adolescentes ≥ 12 anos que requeiram uma dose baixa a média de corticosteroides para inalação em associação com um agonista β_2 de ação prolongada. Se os doentes não estiverem corretamente controlados com 92/22 mcg, a dose pode ser aumentada para 184/22 mcg. Os doentes devem ser regularmente reavaliados. A dose deve ser titulada para a dose mais baixa com a qual é mantido um controlo efetivo dos sintomas. Revinty Ellipta 184/22 mcg deve ser considerado para adultos e adolescentes ≥ 12 anos que requeiram uma dose mais elevada de corticosteroides para inalação em associação com um agonista β_2 de ação prolongada. Os doentes normalmente verificam uma melhoria na função pulmonar 15 minutos após a inalação. É necessário o uso diário regular para manter o controlo dos sintomas de asma e o uso deve ser continuado mesmo quando esta é assintomática. Se os sintomas surgirem no período entre as doses, deve ser utilizado um agonista β_2 de curta duração de ação, de curta duração de ação, para o alívio imediato. A dose máxima recomendada é 184/22 mcg 1x/dia. **Crianças <12 anos** A segurança e a eficácia ainda não foram estabelecidas na indicação para a asma. **DPOC (92/22 mcg)** **Adultos ≥ 18 anos** Uma inalação 1x/dia. Os doentes normalmente verificam uma melhoria na função pulmonar 16-17 minutos após a inalação. **População pediátrica** Não existe utilização relevante na população pediátrica para a indicação de DPOC. **Populações especiais Idosos (> 65 anos)** e **Compromisso renal** Não é necessário ajustar a posologia. **Compromisso hepático** Estudos revelaram um aumento na exposição sistémica ao FF. Devem tomar-se precauções na definição da posologia em doentes com compromisso hepático que possam estar em risco mais elevado de reações adversas sistémicas associadas a corticosteroides. Para os doentes com compromisso hepático moderado ou grave a dose máxima é de 92/22 mcg. **Modo de administração** Via inalatória. Deve ser administrado à mesma hora do dia, todos os dias. Se uma dose for omitida, deve tomar-se a próxima dose à hora habitual no dia seguinte. Após inalação, os doentes devem enxaguar a boca com água sem a engolir. **CONTRAINDICAÇÕES** Hipersensibilidade às substâncias ativas ou a qualquer um dos excipientes. **EFEITOS INDESEJÁVEIS** As reações adversas mais frequentemente notificadas foram cefaleia e nasofaringite. Com a exceção de pneumonia e fraturas, o perfil de segurança foi semelhante em doentes com asma e DPOC. Durante os estudos clínicos, pneumonia e fraturas foram mais frequentemente observadas em doentes com DPOC. **Infeções e infestações** Frequentes Pneumonia, infeção do trato respiratório superior, bronquite, gripe, candidíase da boca e da garganta **Doenças do sistema imunitário** Raras Reações de hipersensibilidade incluindo anafilaxia, angioedema, erupção cutânea e urticária **Doenças do metabolismo e da nutrição** Pouco Frequentes Hiperglicemia **Perturbações do foro psiquiátrico** Raras Ansiedade **Doenças do sistema nervoso** Muito frequentes Cefaleia Raras Tremor **Afeções oculares** Pouco frequentes Visão turva **Doenças cardíacas** Pouco frequentes Extra-sístoles Raras Palpitações, taquicardia **Doenças respiratórias, torácicas e do mediastino** Muito frequentes Nasofaringite Frequentes Dor orofaríngea, sinusite, faringite, rinite, tosse, disfonia Raras Broncospasmo paradoxal **Doenças gastrointestinais** Frequentes Dor abdominal **Afeções musculoesqueléticas e dos tecidos conjuntivos** Frequentes Artralgia, dorralgia, fraturas, espasmos musculares **Perturbações gerais e alterações no local de administração** Frequentes Pirexia. **TITULAR DA AIM** GlaxoSmithKline (Ireland) Limited, 12 Riverwalk, Citywest Business Campus, Dublin 24, Irlanda **DATA DA REVISÃO DO TEXTO** outubro 2021. **APRESENTAÇÃO:** Revinty Ellipta 92 mcg+22 mcg, 30 doses; Revinty Ellipta 184 mcg+22 mcg, 30 doses. **Regime de comparticipação:** Escalão B. Regime Geral 69%; Regime Especial 84%. **Medicamento Sujeito a Receita Médica.** Está disponível informação pormenorizada sobre este medicamento no sítio da internet da Agência Europeia de Medicamentos <http://www.ema.europa.eu/>. Consultar o RCM completo para informação detalhada. Para mais informações e em caso de suspeita de um acontecimento adverso ou de outra informação de segurança, contactar o departamento médico da GlaxoSmithKline - +351 214129500. Para mais informações contactar o representante local do titular da AIM: Bial- Portela & C^o, S.A., -À Av. da Siderurgia Nacional, 4745-457 S.Mamede do Coronado; NIF: 500220913. As Marcas Registradas são propriedade ou licenças das empresas do grupo GSK. ©2022 empresas do grupo GSK ou sob licença.DMGMA_PT211117

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Índice Volume 6 Número 1 Thorax com artigos do Archives of Disease in Childhood janeiro / fevereiro 2022

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1 Editorial

7 Editorial

- 7 Redefinir a linha temporal da tuberculose na perspetiva da BCG
- 8 Uma pandemia respiratória deve focar-se no controlo tabágico

10 Artigo original

- 10 Agregados familiares com crianças e risco de COVID-19: um estudo com mais de 300 000 adultos que vivem em agregados familiares com profissionais de saúde na Escócia
- 16 Exposição a antibióticos durante a gravidez e asma na criança: um estudo de coorte nacional de nascimentos que investiga o tempo de exposição e o tipo de parto

22 Tuberculose

- 22 O tempo de exposição à *Mycobacterium tuberculosis* explica a variação da eficácia da BCG: uma revisão sistemática e meta-análise

31 Exposição ambiental

- 31 Associação entre a função pulmonar de crianças em idade escolar e a exposição a curto prazo a poluição e pólenes na atmosfera: A coorte PARIS
- 37 Os espaços verdes podem melhorar a saúde pulmonar em zonas de poluição atmosférica baixa a moderada mas não alta: Estudo *Seven Northeastern Cities*

42 Fibrose quística

- 42 Doença do vírus sincicial respiratório grave em bebés prematuros: um caso de imaturidade inata



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Editorial

O 1º número de 2022, volume 6, 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, mantém o foco na pandemia, mas gradualmente afasta-se concentrando-se nas doenças não transmissíveis e nas influências do meio ambiente na saúde respiratória.

O Editorial “*Redefinir a linha temporal da tuberculose na perspetiva da BCG*” remete para o artigo “O tempo de exposição a *Mycobacterium tuberculosis* explica a variação da eficácia da BCG: uma revisão sistemática e meta-análise”, em que Trauer e colaboradores analisam a dinâmica da história natural da tuberculose, em interação com a proteção conferida pela vacina BCG, potencialmente transitória.

O 2º Editorial deste número “Uma pandemia respiratória deve focar-se no controlo tabágico” analisa o acréscimo de risco para contrair e maior gravidade da covid19 nos fumadores, para avaliar a oportunidade de determinar medidas mais eficazes do controlo de tabagismo para a sua redução em convergência com a meta de extinção completa de consumo até 2030!

O artigo “Agregados familiares com crianças e risco de COVID-19: um estudo com mais de 300 000 adultos que vivem em agregados familiares com profissionais de saúde na Escócia” é de leitura interessante. É um estudo que decorre entre de 1 de março e 12 de outubro de 2020. Seria interessante perceber se este estudo poderia replicar-se e que resultados obteria nesta altura, em 2021-22...

Cecilie Skaarup Uldbjerg et al, descrevem a associação entre exposição a antibióticos durante a gravidez e asma infantil: neste estudo descreve-se diferentes associações com o trimestre da gravidez da exposição bem como o tipo de parto. Embora especulativo a associação a alteração do microbioma é uma explicação plausível e apetecível.

E por fim 2 estudos que relatam as influências do ambiente na função pulmonar. Estes estudos embora com objetivos e metodologias distintas demonstram a influencia na saúde pulmonar do ar que respiramos.

Boas leituras!

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Redefinir a linha temporal da tuberculose na perspectiva da BCG

David Dowdy

Estima-se que 1,7 mil milhões de pessoas - 23% da população mundial - tenham sido infetadas pela *Mycobacterium tuberculosis*.¹ Há mais de cinco décadas que se sabe que, uma vez que alguém tenha sido infetado pela *M. tuberculosis* durante um período de 2-5 anos, o risco de desenvolver tuberculose (TB), salvo uma segunda infecção ou condição imunossupressora, é reduzido.² No entanto, várias publicações e manuais continuam a citar uma “regra de ouro” segundo a qual 5% das pessoas com infecção latente por TB desenvolverão TB reativada após este período inicial de alto risco. Várias análises recentes reafirmaram que este número de 5% é uma estimativa substancialmente exagerada.³⁻⁴ Nesta edição da *Thorax* com artigos do ADC, Trauer *et al.* reanalisam dados de 21 ensaios clínicos de vacinação contra o bacilo Calmette-Guérin (BCG).⁵ A sua análise abrangente fornece mais uma prova de que a tradicional conceptualização da infecção por TB latente - como um estado estático em que as pessoas experimentam um risco de reativação constante e razoavelmente elevado durante muitos anos, se não mesmo décadas, deve ser abandonada.

Análises anteriores estimaram que a proteção derivada da BCG contra a doença da tuberculose poderá diminuir após cerca de 10 anos,⁶ embora sejam escassos os dados de seguimento. Trauer *et al.* atualizaram uma meta-análise anterior dos ensaios da vacina BCG⁷ para explorar se esta diminuição da proteção reflete muito provavelmente o tempo de exposição à *M. tuberculosis* ou o tempo de reativação da doença. Alcançaram este objetivo separando os ensaios em duas categorias de carga subjacente à tuberculose (carga decrescente vs. carga elevada contínua) e de duração do seguimento (precoce vs. tardio). Com esta classificação, os resultados sugerem que a BCG pode ter proporcionado níveis elevados de proteção durante o seguimento precoce em todos os ensaios - proteção que pode ter persistido em cenários de diminuição da carga de TB (ou seja, onde o risco de exposição repetida foi reduzido). Mas, em cenários de elevada transmissão contínua de tuberculose - onde o risco de infecção contínua superou o risco de reativação tardia - a BCG pode ter oferecido pouca (ou mesmo nenhuma) proteção a longo prazo. Esta descoberta é consistente com a hipótese de que a diminuição observada da proteção BCG reflete o tempo de exposição e não o tempo de reativação.

Embora represente apenas uma das muitas explicações razoáveis para os dados observados, esta hipótese é bela na sua sim-

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plicidade; não precisa de invocar relações mais complexas, como com a latitude ou micobactérias não tuberculosas.⁷ Conforme observado pelos próprios autores, esta descoberta não é surpreendente, dada a baixa taxa de reativação ‘tardia’ (ou seja, progressão para a doença da tuberculose para além do período de alto risco inicial de 2-5 anos após a infecção inicial). Em cenários de elevada carga contínua de tuberculose, a incidência de tuberculose doença durante períodos de seguimento mais longos reflete largamente eventos de infecção repetidos, em vez de reativação tardia. Por exemplo, o risco anual de infecção por TB na Índia na altura do maior ensaio BCG (1,4%-1,7%) é semelhante ao risco remanescente de tuberculose ao longo da vida (1,2%-2,2%) para um adulto normal infetado com TB que vive num ambiente de exposição reduzida, como é o caso dos EUA atualmente.

Mas as descobertas de Trauer *et al.* são difíceis de conciliar com o pensamento tradicional de que, uma vez que alguém é infetado com *M. tuberculosis*, essa pessoa irá experimentar um risco persistentemente elevado de reativação da doença. Se este fosse o caso (assumindo que uma elevada fração de pessoas nos ensaios BCG foram expostas à TB nos anos que se seguiram à vacinação), a proteção a longo prazo proporcionada pela BCG não dependeria fortemente da carga de TB na comunidade envolvente. É importante interpretar estas conclusões à luz de duas importantes ressalvas. Em primeiro lugar, nos ensaios realizados principalmente com adultos, o resumo da previsão de Trauer *et al.* sobre o efeito da BCG ao longo de um seguimento mais longo não variou muito entre cenários de carga contínua (taxa de incidência 1,05, 95% IC 0,87 a 1,24) e cenários de carga decrescente (0,86, 95% IC 0,57 a 1,15). Em segundo lugar, nos ensaios realizados maioritariamente com crianças, apenas um foi categorizado pelos autores como “carga contínua, seguimento mais prolongado”.

Este ensaio foi realizado em Porto Rico por volta de 1950 - numa altura em que a mortalidade por TB decaiu mais de 90% nas duas décadas seguintes.⁸ Se este estudo fosse reclassificado como estando numa região de ‘declínio’ de carga, o peso das evidências que apoiam a principal conclusão dos autores seria correspondentemente mais leve. Apesar destas ressalvas - e independentemente das conclusões finais - esta nova análise proporciona um enorme valor científico acrescido, sob a forma de (1) descrições detalhadas de cada ensaio que contribuem para a análise; (2) reavaliação rigorosa (e, em alguns casos, revisão) de resultados anteriormente publicados; (3) inclusão de novos dados desagregados do ensaio de maior contribuição para a análise e (4) publicação aberta de todos os

dados e códigos, incluindo para a criação de importantes indicadores. Estas descrições, análises e dados adicionais servirão como um recurso inestimável para uma comunidade científica que continuará, sem dúvida, a debater o mecanismo de ação da BCG durante décadas.

Nos últimos 5 anos, concentrámo-nos na linha temporal da infecção por tuberculose. A história natural da tuberculose é agora reconhecida como uma entidade dinâmica que é melhor caracterizada não como um risco de “reativação” elevado e persistente, mas como um equilíbrio temporal variável entre a atividade do patógeno (que diminui rapidamente nos primeiros anos após a infecção inicial) e a resposta do hospedeiro (que pode ser enfraquecida a qualquer momento) - um equilíbrio em que tanto a intensidade como a frequência da infecção também desempenham um papel importante. Trauer *et al.* acrescentam a esta compreensão da história natural da tuberculose, observando as evidências de uma perspectiva diferente: a da proteção induzida pela BCG ao longo do tempo. As suas descobertas têm implicações importantes para o desenvolvimento de novas vacinas contra a tuberculose, que também podem necessitar de uma proteção a longo prazo contra a infecção (e a progressão para a doença) para que possam ter um impacto sustentado a nível populacional. Também sublinham a centralidade da redução do peso da transmissão da tuberculose no caso de querermos pôr fim a esta epidemia que rouba a vida a mais de um milhão de pessoas todos os anos.

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Uma pandemia respiratória deve focar-se no controlo tabágico

Anthony A Laverty, Christopher Millett

A ideia de que o tabaco pode proteger contra a COVID-19 foi sempre uma ideia improvável. Ambos causaram perdas terríveis de vidas humanas. Até 13 de julho de 2021, a COVID-19 matou cerca de 4 milhões de pessoas, enquanto que o tabaco mata 8 milhões de pessoas todos os anos.^{1,2} No entanto, algumas evidências recentes sugerem que o consumo de tabaco esteja associado a um risco reduzido de infecção e morte por COVID-19. Esta descoberta gerou, sem surpresa, um interesse substancial entre os investigadores, na classe médica e no público.³

O artigo de Clift *et al*⁴ editado na edição original da *Thorax* apresenta o nosso entendimento das ligações entre o tabagismo e a COVID-19 ao combinar uma série de dados relevantes para empregar duas abordagens analíticas distintas. No seu conjunto, estas análises sugerem um papel causal do tabagismo na gravidade da COVID-19. Os autores ligaram dados do UK Biobank com registos de cuidados primários, dados de testes de COVID-19, internamentos hospitalares e certidões de óbito para realizar análises de observação e de aleatorização mendeliana. Os dados de testes de COVID-19 só estiveram disponíveis até agosto de 2020, o que significa que os dados sobre infecções confirmadas se concentraram nos estabelecimentos hospitalares. As análises observacionais não concluíram que os atuais fumadores tivessem maior probabilidade de ter infecção por COVID-19 confirmada, mas que tinham maior probabilidade de ser hospitalizados e de morrer da doença. Por exemplo, nos modelos ajustados por idade e sexo, os atuais fumadores apresentaram maior probabilidade de hospitalização comparativamente aos não fumadores (OR 2,19, 95% IC 1,63 a 2,92) e de morrerem de COVID-19 (OR 5,93 (95% IC 4,22 a 8,33)). As análises de aleatorização mendeliana só foram possíveis entre os participantes britânicos brancos, mas estas descobriram que o aumento da propensão genética para começar a fumar esteve associado a uma maior probabilidade de infecção e hospitalização por COVID-19. Foram encontrados riscos acrescidos de infecção, hospitalização e morte com o aumento do peso do tabagismo geneticamente previsto.

O estudo Clift confirma estudos anteriores do Reino Unido de base populacional, incluindo o UCL COVID-19 Social Study, o ZOE COVID-19 Symptom Study e os dados OpenSAFELY.⁵⁻⁷ A análise dos dados da aplicação ZOE até abril de 2020 avaliou a relação do tabagismo com a COVID-19, utilizando sintomas autorreportados para classificar as pessoas como casos suspeitos.⁶ Nos modelos ajustados à idade e sexo, os atuais fumadores apresentaram maior probabilidade de revelar os sintomas clássicos da COVID-19 de febre, tosse de novo e persistente e falta de ar. As associações para reportar um maior número de sintomas, um marcador da gravidade da doença, foram ainda superiores. As análises dos dados OpenSAFELY, que cobrem os registos de cuidados primários de 40% da população do Reino Unido ligados a mais de 10 000 mortes por COVID-19, constataram igualmente que tanto os antigos como

os atuais fumadores eram mais suscetíveis de morrer de COVID-19.⁷ O que estes dois estudos têm em comum é que os resultados e as interpretações diferem entre modelos estatísticos ajustados apenas para idade e sexo e aqueles que são ajustados para um conjunto mais amplo de fatores, como as condições pré-existent. As análises ajustadas a fatores que incluem doenças diagnosticadas revelaram que os fumadores se encontravam em menor risco de COVID-19 do que os nunca fumadores, reforçando a ideia de que fumar poderia ter um efeito protetor. Contudo, a equipa OpenSAFELY realizou análises *post hoc* da sua descoberta de que os fumadores pareciam ter menos probabilidade de morrer de COVID-19, o que deu confiança de que esta aparente diminuição do risco é incorreta. Esta aparente associação foi motivada pelo viés de colisão, onde o ajuste excessivo em modelos totalmente ajustados produz resultados falsos. Mais concretamente, as análises *post hoc* constataram que o efeito protetor do fumo era impulsionado principalmente pelo ajustamento para doenças respiratórias crónicas, significando que estas análises estavam a ajustar uma das principais vias pelas quais o tabagismo aumenta os riscos. O estudo Clift aumenta a nossa confiança na afirmação de que o tabagismo não protege contra a COVID-19, uma vez que as análises de aleatorização mendeliana são menos suscetíveis de confundir do que os estudos observacionais anteriores. Adicionalmente, uma *Living Review* das atuais evidências favorece uma associação entre o tabagismo atual e a gravidade da COVID-19, enquanto evidências recentes do estudo REACT (*Real Time Assessment of Community Transmission*) concluem que o tabagismo está associado a uma maior probabilidade de sintomas persistentes 12 semanas após infecção por COVID-19 (a chamado COVID-19 prolongada)^{8,9}. Em conjunto, todas estas evidências reforçam a existência de impactos negativos do tabagismo nos resultados de COVID-19.

Uma pandemia respiratória deve ser o momento ideal para centrar o pensamento coletivo no controlo do tabagismo. Um novo Plano de Controlo Tabágico em Inglaterra deverá ser publicado ainda este ano, o que representa uma oportunidade para redobrar os nossos esforços. Em abril, o *Royal College of Physicians* (RCP) assinalou quase 60 anos desde o influente relatório *Smoking and Health* com um novo conjunto de recomendações para combater o flagelo do tabagismo.¹⁰ Embora o declínio do tabagismo nos últimos 60 anos seja uma prova dos esforços anteriores, o relatório de 1962 observou que “tanto os costumes sociais como a pressão comercial ultrapassam a voz da precaução” e isto continua a ser verdade até aos dias de hoje.¹¹ O último relatório do RCP constata que o Reino Unido não está no bom caminho para atingir os objetivos de manter o tabagismo a uma taxa inferior a 5% até 2030 e que o tabagismo continua a ser uma das principais causas de desigualdade na saúde. O relatório oferece 10 recomendações para corrigir esta situação. Estas incluem novas medidas como o aumento da idade legal de venda para 21 anos e aumentos anuais na tributação, bem como ações para reduzir a diferença de preços entre os cigarros e o tabaco de enrolar. São necessários mais esforços para apoiar a cessação do tabagismo, combinando o tratamento não obrigatório da dependência tabágica no Serviço Nacional de Saúde com apoio persona-

lizado para alguns grupos. Os grupos destacados para uma atenção específica incluem os que se encontram em condições socioeconómicas mais carenciadas, indivíduos LGBT e indivíduos que sofram de doenças mentais graves. Finalmente, o RCP recomenda limitar as oportunidades para a indústria tabaqueira explorar as lacunas na regulamentação. A indústria tabaqueira tem uma longa história na realização de tais esforços, que continuam até aos dias de hoje, incluindo recentemente, utilizar a inovação de produtos para contornar a proibição dos cigarros de mentol de 2020. Estes esforços devem ser impedidos e a indústria tabaqueira deve ser excluída de toda a criação de políticas se se pretende que o Reino Unido extinga completamente o consumo de tabaco até 2030.

No seu conjunto, as medidas recomendadas no relatório do RCP oferecem uma oportunidade de fazer história no tabagismo e uma pandemia respiratória é o momento perfeito para o fazer.

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ARTIGO ORIGINAL

Agregados familiares com crianças e risco de COVID-19: um estudo com mais de 300 000 adultos que vivem em agregados familiares com profissionais de saúde na Escócia

Rachael Wood,^{1,2} Emma Thomson,³ Robert Galbraith,⁴ Ciara Gribben,¹ David Caldwell,⁵ Jennifer Bishop,⁵ Martin Reid,¹ Anoop S V Shah,⁶ Kate Templeton,⁷ David Goldberg,⁵ Chris Robertson,⁵ Sharon J Hutchinson,⁵ Helen M Colhoun,^{5,8} Paul M McKeigue,² David A McAllister^{5,9}

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RESUMO

Objetivo As crianças estão relativamente protegidas da COVID-19 devido a uma série de mecanismos potenciais. Investigámos se o contacto com crianças também proporciona aos adultos um grau de proteção contra a COVID-19.

Desenho Estudo de coorte baseado em dados administrativos relacionados.

Local Escócia.

População do estudo Todos os profissionais de saúde do Serviço Nacional de Saúde Escocês e seus agregados familiares até março de 2020.

Principal exposição Número de crianças (0–11 anos) a residir no agregado familiar do participante.

Principais resultados Infecção por COVID-19 com necessidade de hospitalização e qualquer infecção por COVID-19 (qualquer teste positivo para SARS-CoV-2) em adultos com ≥18 anos entre 1 de março e 12 de outubro de 2020.

Resultados 241266, 41198, 23783 e 3850 adultos partilharam um agregado familiar com 0, 1, 2 e 3 ou mais crianças, respetivamente. Ao longo do período de estudo, o risco de COVID-19 com hospitalização foi progressivamente reduzido com um número crescente de crianças - HR totalmente ajustado (aHR) 0,93 por criança (95% IC 0,79 a 1,10). O risco de qualquer infecção por COVID-19 foi reduzido de forma semelhante, sendo a associação estatisticamente significativa (aHR por criança 0,93; 95% IC 0,88 a 0,98). Após a reabertura das escolas a todas as crianças em agosto de 2020, não foi observada qualquer associação entre a exposição a crianças e o risco de qualquer infecção por COVID-19 (aHR por criança 1,03; 95% IC 0,92 a 1,14).

Conclusão Entre março e outubro de 2020, residir com crianças esteve associado a um risco atenuado de qualquer infecção por COVID-19 e COVID-19 com hospitalização entre adultos a residir em agregados familiares com profissionais de saúde. Não se observaram evidências de que residir com crianças aumentasse o risco de infecção por COVID-19 em adultos, inclusivamente durante o período após a reabertura das escolas.

INTRODUCTION

To date, children seem to be relatively protected from SARS-CoV-2, being substantially less likely to develop symptomatic infection (COVID-19) or serious illness.^{1–2} Zimmermann and Curtis³ recently provided a wide-ranging review of the proposed mechanisms for this difference, including factors such as concurrent infections competing with SARS-CoV-2 in vivo, differences in microbiota, higher melatonin levels and lower intensity of exposure to SARS-CoV-2. Differences in innate and/or acquired immune mechanisms may play a part,^{3–11} as might increased innate immune responsiveness following vaccinations and high exposure to respiratory viruses.^{3,12} Alternatively, pre-exposure to antigenically similar

What is already known on this topic?

- Young children are less likely to develop COVID-19 and severe COVID-19 than adults, due to a range of potential mechanisms, among which is the possibility of cross-protective immunity to SARS-CoV-2.
- Whether contact with young children offers adults a degree of protection from COVID-19 or not is unknown.
- Two studies have suggested that contact with children may be inversely associated with COVID-19 risk (risk of infection, hospitalisation or death from COVID-19).

What this study adds?

- In our large cohort, adults with young children were at lower risk of testing positive for SARS-CoV-2 and possibly also of developing COVID-19 requiring hospitalisation.
- Adults living with young children were not at increased risk of COVID-19, including during August–October 2020 when nurseries and schools were open for all children.
- Verification of this finding is warranted in other populations and settings.

infectious agents (providing subsequent specific cross-protection against SARS-CoV-2) may also be relevant. Children have higher levels of exposure to endemic coronaviruses than adults.^{13–14} Evidence exists for B cell and T cell cross-reactivity between SARS-CoV-2 and endemic coronaviruses,^{4–10} and SARS-CoV-2 responsive T cells have been shown to provide protection against COVID-19.¹¹

Given this latter possibility, we reasoned that adults who are close contacts of children might also be protected from SARS-CoV-2 due to a degree of immune cross-protection. This could be relevant to decisions to close nurseries and schools in response to SARS-CoV-2¹⁵ and teachers' perceptions of workplace safety.¹⁶ Since few relevant studies exist,¹⁷ we used a recently reported cohort of around 160 000 healthcare workers and 250 000 household members in Scotland¹⁸ to test the hypothesis that risk of COVID-19 in adults is attenuated among those living with young children.

Table 1 Characteristics of adult household contacts by number of young children

	0 children aged 0–11 years	1 child aged 0–11 years	2 children aged 0–11 years	3+ children aged 0–11 years
Number of adults	241 266	41 198	23 783	3850
Adults who are healthcare workers	121 004 (50.15)	22 025 (53.46)	13 179 (55.41)	2237 (58.10)
Age, mean (SD)	44.53 (15.04)	39.82 (10.58)	39.01 (8.08)	38.47 (6.62)
Male	105 116 (43.57)	17 639 (42.82)	10 803 (45.42)	1783 (46.31)
Scottish index of multiple deprivation				
1 – most deprived	37 242 (15.44)	5655 (13.73)	2447 (10.29)	373 (9.69)
2	46 147 (19.13)	7700 (18.69)	3599 (15.13)	582 (15.12)
3	48 659 (20.17)	7491 (18.18)	4280 (18.00)	706 (18.34)
4	52 847 (21.90)	9701 (23.55)	6102 (25.66)	999 (25.95)
5 – least deprived	56 371 (23.36)	10 651 (25.85)	7355 (30.93)	1190 (30.91)
Race/ethnicity – non-white	7768 (3.22)	2088 (5.07)	1162 (4.89)	259 (6.73)
Comorbidity count				
None	207 796 (86.13)	37 315 (90.57)	21 924 (92.18)	3564 (92.57)
One	24 897 (10.32)	3231 (7.84)	1579 (6.64)	252 (6.55)
Two or more	8573 (3.55)	652 (1.58)	280 (1.18)	34 (0.88)
Occupation of healthcare worker in household				
Nursing and midwifery	102 514 (42.49)	18 688 (45.36)	10 085 (42.40)	1530 (39.74)
Administrative services	44 929 (18.62)	6710 (16.29)	3236 (13.61)	404 (10.49)
Support services	27 294 (11.31)	3232 (7.85)	1386 (5.83)	236 (6.13)
Medical and dental	20 836 (8.64)	4326 (10.50)	3586 (15.08)	849 (22.05)
Allied health profession	20 007 (8.29)	3798 (9.22)	2974 (12.50)	442 (11.48)
Other	25 686 (10.65)	4444 (10.79)	2516 (10.58)	389 (10.10)
Occupational role of healthcare worker in household				
Non-patient facing	50 441 (20.91)	7453 (18.09)	3667 (15.42)	471 (12.23)
Patient facing	137 697 (57.07)	25 461 (61.80)	15 485 (65.11)	2627 (68.23)
Undetermined	53 128 (22.02)	8284 (20.11)	4631 (19.47)	752 (19.53)
Part time working in healthcare worker in household				
Whole time	147 608 (61.18)	19 482 (47.29)	8296 (34.88)	1206 (31.32)
Part time	88 351 (36.62)	20 329 (49.34)	14 183 (59.64)	2281 (59.25)
Not recorded	5307 (2.20)	1387 (3.37)	1304 (5.48)	363 (9.43)
Tested for SARS-CoV-2	14 736 (6.11)	2835 (6.88)	1823 (7.67)	354 (9.19)

Statistics are the number (percentage) of adults with each characteristic except for age, which is given as the mean and SD.

METHODS

Population, data sources and record linkage

Full details of the population studied are reported elsewhere.¹⁸ In brief, we identified healthcare workers in Scotland (aged 18–65 years) using databases including all individuals directly employed by the National Health Service (NHS) and all general practitioners providing services to the NHS, as of March 2020. We identified other members of the healthcare workers' households (all ages) using the NHS Scotland master patient index and exact address matching. We linked these data to multiple Scotland-wide databases indicating virology testing for SARS-CoV-2, hospitalisation, critical care admission and death. Exposure, covariate and outcome data were examined for all adults aged ≥18 years living in a healthcare worker household. All data from 1 March to 12 October 2020 were included.

Outcome

The primary outcome was COVID-19 requiring hospitalisation, defined as a first positive PCR test¹⁹ for SARS-CoV-2 up to 28 days prior to, or during, a hospital admission. Secondary outcomes were any COVID-19 (defined as any positive test for SARS-CoV-2) and severe COVID-19 (defined as a positive test for SARS-CoV-2 up to 28 days prior to admission for critical care or death).

Exposure

The primary exposure was the number of young children (aged 0–11 years) in each household. Additional analyses examined risk by the number of preschool children (aged 0–4 years), primary school children (aged 5–11 years), older children (aged 12–17 years) and other adults (aged ≥18 years).

Covariates

Data on age, sex and deprivation (Scottish Index of Multiple Deprivation quintile) were obtained from the linked databases. Prespecified comorbidities (see online supplemental table S1) were defined using previous hospitalisation and prescribing data. Ethnicity was imputed from forename and surname using the ONOMAP algorithm.²⁰

Occupational covariates were defined at the household level based on the characteristics of the member who was a healthcare worker. These included the healthcare worker's occupation (eg, medical and nursing), potential exposure to SARS-CoV-2 (eg, patient-facing role or not), seniority, length of service, immigration status and full/part-time working status. In households with more than one healthcare worker, the highest risk category was applied. A causal diagram showing the assumed relation between these covariates is provided in online supplemental figure S1.

Statistical analysis

We plotted the cumulative incidence of hospitalisation for COVID-19 among adults according to the number of young children in each household. We modelled COVID-19 requiring hospitalisation, any COVID-19 and severe COVID-19 using Cox regression, calculating robust SEs to allow for clustering due to shared household membership and stratifying on groups of health board areas to allow for differences in baseline hazard. We present effect estimates for minimal models adjusting for age, full models including all covariates and intermediate models. We conducted a range of sensitivity analyses incorporating additional covariates and/or restricting the population examined.

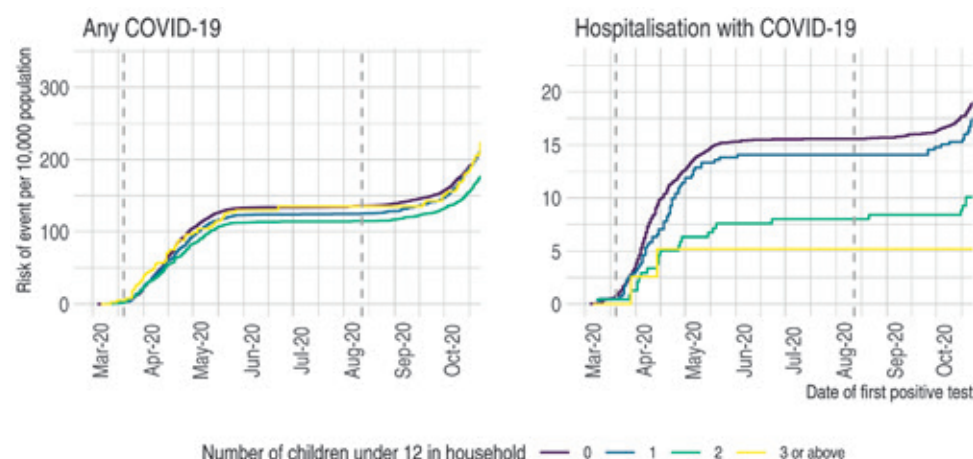


Figure 1 Risk of any COVID-19 and of COVID-19 requiring hospitalisation in adults living in healthcare worker households by number of young children (aged 0–11 years) cumulative incidence (risk) plots of any COVID-19 and of COVID-19 requiring hospitalisation by number of young children (aged 0–11 years) in household. Vertical dotted lines indicate dates when schools closed and reopened.

RESULTS

Of the 310 097 adults living in a healthcare worker household, 241 266 (78%), 41 198 (13%), 23 783 (7.8%) and 3850 (1.2%) shared their household with 0, 1, 2 and 3 or more young children, respectively. Compared with adults living with no young children, those living with children were on average 5 years younger; were less likely to live in the most deprived areas; were more likely to be of non-white ethnicity; and were less likely to have comorbidities (table 1 and online supplemental table S1). Adults living with young children were slightly more likely to be tested for SARS-CoV-2 (table 1).

Household composition differed according to the number of young children. Households with more children were more likely to include two or more adults. More than a quarter of adults who shared a household with a single child under 11 years also shared a household with a child aged 12–17 years (online supplemental table S2).

COVID-19 requiring hospitalisation

Compared with adults living with no young children, the risk of COVID-19 requiring hospitalisation was reduced in those living with children (figure 1). The unadjusted HR for COVID-19 requiring hospitalisation was 0.77 per each additional young child in the household (95% CI 0.65 to 0.90, table 2). On adjusting for adults' age, this association was attenuated (HR per child 0.88; 95% CI 0.75 to 1.04). Further smaller changes were seen after adjusting for other potential confounders (sex, deprivation, occupation, professional role, staff/non-staff status, length of service, number of adolescents and adults in household, comorbidity count plus selected comorbidities (see online supplemental table S1) and full-time/part-time working status), with the fully adjusted HR (aHR) being 0.93 per child (95% CI 0.79 to 1.10).

Similar associations were found when analysis was restricted to households where at least one adult was a patient-facing healthcare worker (aHR per child 0.89; 95% CI 0.74 to 1.08, online supplemental table S3). In a stratified analysis, the aHR per child was 1.11 (95% CI 0.92 to 1.35) for adults living in households containing a full-time healthcare worker and 0.65 (95% CI 0.46 to 0.91) for those in households containing a part-time healthcare worker (p interaction=0.002). A further stratified analysis found similar results for adults with (aHR 0.91; 95% CI 0.75 to 1.10) and without (aHR 0.90; 95% CI 0.65 to 1.25) any comorbidities.

Any COVID-19

Compared with adults living with no young children, the risk of any COVID-19 was reduced in those living with children (figure 1). In the full study population, the fully aHR for any COVID-19 was similar to that seen for COVID-19 requiring hospitalisation but with narrower CIs (aHR per child 0.93 95% CI 0.88 to 0.98, table 3). The inverse association between number of young children in the household and risk of any COVID-19 was similar in adults with (aHR per child 0.84; 95% CI 0.72 to 0.99) and without (aHR 0.93; 95% CI 0.88 to 0.97) comorbidities and in adults living in households containing a full-time (aHR 0.95; 95% CI 0.88 to 1.02) and part-time (aHR 0.88; 95% CI 0.81 to 0.94) healthcare worker (p interaction=0.44).

Further analysis of the risk of any COVID-19 revealed stronger inverse associations for adults living with preschool children, compared with those living with primary school children, adolescents or other adults (table 4). Similar differences between the age groups, but with wider 95% CIs reflecting the smaller numbers of events, were also found for the primary outcome of COVID-19 requiring hospitalisation (online supplemental table S4).

Table 2 Risk and HRs for COVID-19 requiring hospitalisation for adults living in healthcare worker households by number of young children

	No children aged 0–11 years	1 child aged 0–11 years	2 children aged 0–11 years	3+ children aged 0–11 years	Per child
N adults with COVID-19 requiring hospitalisation	390	59	20	2	–
Total N adults	241 266	41 198	23 783	3850	–
Risk per 10 000	16.2	14.3	8.4	5.2	–
Unadjusted	1	0.89 (0.67–1.17)	0.53 (0.34–0.83)	0.33 (0.08–1.32)	0.77 (0.65–0.90)
Model 1	1	1.09 (0.82–1.46)	0.69 (0.44–1.10)	0.44 (0.11–1.79)	0.88 (0.75–1.04)
Model 2	1	1.11 (0.83–1.49)	0.72 (0.45–1.15)	0.44 (0.11–1.78)	0.90 (0.76–1.05)
Model 3	1	1.13 (0.84–1.52)	0.73 (0.46–1.16)	0.45 (0.11–1.81)	0.90 (0.77–1.06)
Model 4	1	1.17 (0.87–1.57)	0.78 (0.49–1.26)	0.49 (0.12–1.97)	0.93 (0.79–1.10)

HRs obtained from Cox proportional hazard models. Model 1 adjusts for adults' age using a penalised spline function. Model 2 additionally adjusts for sex, Scottish Index of Multiple Deprivation quintile, occupation (eg, nursing and medical), occupational role (patient facing, non-patient facing and undetermined), healthcare worker (yes/no), length of service, number of children aged 12–17 years in household and number of adults in household. Model 3 additionally adjusts for the comorbidity count and specific conditions (ischaemic heart disease, other heart disease, other circulatory system diseases, advanced chronic kidney disease, asthma and chronic lower respiratory disease, neurological disorders, decompensated liver disease, any immunological condition, malignant neoplasms, disorders of oesophagus, stomach and duodenum, type 1 diabetes and type 2 diabetes). Model 4 additionally adjusts for part-time status (additional model not prespecified).

Table 3 Risk and HRs for any COVID-19 for adults living in healthcare worker households by number of young children

	No children aged 0–11 years	1 child aged 0–11 years	2 children aged 0–11 years	3+ children aged 0–11 years	Per child
N adults with any COVID-19	4324	717	345	65	–
Total N adults	241 266	41 198	23 783	3850	–
Risk per 10 000	179.2	174.0	145.1	168.8	–
Unadjusted	1	0.97 (0.89–1.06)	0.81 (0.72–0.92)	0.96 (0.73–1.26)	0.93 (0.89–0.98)
Model 1	1	0.93 (0.85–1.02)	0.78 (0.69–0.88)	0.91 (0.69–1.21)	0.91 (0.87–0.96)
Model 2	1	0.93 (0.85–1.02)	0.79 (0.70–0.90)	0.90 (0.68–1.18)	0.92 (0.87–0.96)
Model 3	1	0.93 (0.85–1.02)	0.79 (0.70–0.90)	0.90 (0.68–1.18)	0.92 (0.87–0.96)
Model 4	1	0.94 (0.86–1.03)	0.82 (0.72–0.93)	0.92 (0.70–1.22)	0.93 (0.88–0.98)

Model fitting and covariates as per footnote of table 2.

School reopening

Nurseries and schools in Scotland were closed from 23 March 2020 until the end of the school year in late June 2020. They reopened to all children on 12 August 2020 and remained open until the Christmas holiday. Some childcare or school provision was available for children of key workers, including healthcare workers, between March and August. Between 12 August and 12 October 2020 (the end date of our study period), there were an additional 1337 cases of any COVID-19 and 20 cases of COVID-19 requiring hospitalisation among the adults in our study population (figure 1), and the aHR for any COVID-19 was 1.03 (95% CI 0.92 to 1.14) per each additional young child in the household.

Additional analyses

Results for the much less common outcome, severe COVID-19, are shown in online supplemental tables S5 and S6). Restricting the analysis to individuals identified via the ONOMAP algorithm as white did not modify the associations for any of the outcomes. The full set of regression coefficients and SEs for all fitted models are provided at <https://github.com/ChronicDiseaseEpi/hcw>.

DISCUSSION

We examined the hypothesis that the risk of COVID-19 in adults is attenuated for those with high exposure to young children (0–11 years) due to presumed cross-protective immunity. Among a cohort of over 300 000 adults living in a household with a healthcare worker in Scotland, the risk of testing positive for SARS-CoV-2 over the period March–October 2020 was slightly lower for individuals living with young children, and this reduction persisted after adjusting for potential confounders. The risk of COVID-19 requiring hospitalisation (primary outcome specified) was also lower for those living with young children, although this finding did not reach statistical significance.

Very few studies have directly examined whether contact with children affords adults protection from SARS-CoV-2. Prior to our recent preprint,²¹ only one study touching on this question was identified.¹⁷ In this German study, 1186 of 4010 patients who had recovered from COVID-19 responded to a survey. The proportion of individuals reporting regular contact with children aged <11 years was lower than expected based on general population rates. More recently a preprint has been published by the OpenSAFELY group.²² In this general population sample of 12 million people in England, between February and July 2020, adults sharing a household with children aged 0–11 years were not at increased risk of any COVID-19 or COVID-19 requiring hospitalisation or critical care admission and were at a lower risk of death from COVID-19.

These studies are congruent with our findings and together suggest that, to date, exposure to young children is not associated with increased risk of COVID-19 in adults but with a small reduction in risk.

The risk to adults of COVID-19 will presumably reflect a dynamic balance between the risk that children may directly transmit SARS-CoV-2 to their adult contacts, and the possibility that they may enhance cross-protective immunity through prior transmission of other respiratory viruses. In this context, our current study provides reassurance that adults sharing a household with young children remained at no increased risk of COVID-19 during August–October 2020 when schools were reopened and community transmission of SARS-CoV-2 was occurring.

This study has some limitations. The observed inverse association between living with young children and adults' risk of COVID-19 was not strong and could be a chance finding. Our primary outcome, COVID-19 requiring hospitalisation, was uncommon; hence, HR CIs were wide. Although we suspected that statistical power would be limited, we prespecified this as the primary outcome as we were concerned that high rates of (non-SARS-CoV-2) acute respiratory infection in households with small children might have led to higher levels of testing for SARS-CoV-2 and hence biased ascertainment of any COVID-19. The level of testing was indeed higher among those adults who shared a household with young children. However, point estimates for COVID-19 requiring hospitalisation and any COVID-19 were similar, and for the latter, they were statistically significant.

Another possibility is that, despite extensive adjustment for potential confounders, the observed inverse association may be the result of residual confounding. On stratified analyses, the inverse association was evident for adults living in part-time healthcare worker households but less obvious for those living in full-time healthcare worker households. Since part-time workers with increasing numbers of children likely work fewer hours (and therefore have lower occupational exposure to SARS-CoV-2), and since we lacked accurate data on hours worked during the pandemic, we cannot exclude the possibility of unmeasured confounding as a cause of the observed associations.

It has been suggested that the inverse association between living with young children and risk of COVID-19 may result from adults living with children spending less time outside the home in settings in which SARS-CoV-2 may be transmitted. While plausible, we are unable to find any empirical evidence in support of this view. For much of the pandemic across much of Scotland, many social venues have been closed. In addition, evidence from the 2018 Scottish Household Survey (online supplemental tables

Table 4 HRs for any COVID-19 for adults living in healthcare worker households by number of persons of different ages

	Per child aged 0–4 years	Per child aged 5–11 years	Per child aged 12–17 years	Per adult aged 18 years or above
Unadjusted	0.88 (0.81–0.96)	1.07 (1.01–1.13)	0.87 (0.84–0.89)	0.97 (0.91–1.02)
Model 1	0.87 (0.80–0.95)	1.03 (0.97–1.09)	0.88 (0.85–0.90)	0.94 (0.88–0.99)
Model 2	0.86 (0.78–0.94)	1.05 (0.99–1.12)	1.04 (1.02–1.07)	0.95 (0.89–1.01)
Model 3	0.86 (0.78–0.94)	1.05 (0.99–1.12)	1.04 (1.02–1.07)	0.95 (0.89–1.01)
Model 4	0.87 (0.80–0.95)	1.06 (1.00–1.12)	1.04 (1.01–1.07)	0.96 (0.90–1.02)

Model specification and covariates as per footnote of table 2. The unadjusted models and model 1 were fitted separately for each exposure (eg, aged 0–5 years and aged 6–11 years), but all exposures were included in models 2, 3 and 4. The effect estimates corresponds to 'per child' column in tables 2–3, where the counts of children and adults were treated as continuous variables, which assumes that any association between the number of children (or adults) and the hazard rate is log-linear.

S7–10) suggests that adults who live with young children are as likely to visit restaurants and gyms as other adults of the same age and indeed more likely to visit places of worship and cinemas.

If a protective effect of children on COVID-19 rate and severity in their adult contacts is confirmed, this could involve cross-reactive immunity to endemic COVID-19 infections acquired outside the home, for example, at nursery or school. First, evidence of antigenic similarity between N proteins of SARS-CoV-2 and those of endemic beta coronaviruses (strains Cov-OC43 and Cov-NL63) has now been shown in studies of cell-mediated immunity. There is also evidence of cross-reactivity in antibody-mediated immunity, although it is uncertain how well this protects against COVID-19.^{4–10} Second, respiratory samples obtained from children during investigation for respiratory tract infections show high levels of seasonal, endemic coronaviruses.^{13,14} Third, as well as having higher rates of exposure to such viruses, children may transmit seasonal coronaviruses to their household contacts. Younger adults (aged 15–44 years) that include those most likely to live with young children have higher levels of antibodies to N proteins of CoV-OC43 than do older adults,²³ although whether this reflects exposure at home via contact with children, or elsewhere, is unknown. It would be important to compare the prevalence of antibodies to SARS-CoV-2, and to seasonal coronaviruses, in those with and without substantive exposure to children of different age groups.

Moreover, it is important to note that differences in previous exposure to other coronaviruses is only one of a range of potential explanations for the difference in COVID-19 risk among adults and children, for most of which—such as differences in melatonin levels—there are no plausible mechanisms by which adult contacts could share in the protection.³

Notwithstanding possible mechanisms, our findings provide sufficient evidence of a potentially interesting protective effect against COVID-19 for adults living with young children to warrant further study in other populations (eg, adults working in nurseries and primary schools) and settings. Ongoing work to explore whether potential protective effects persist as community transmission patterns evolve, in particular in response to the emergence of new viral strains and the implementation of vaccination programmes, would also be beneficial.

CONCLUSION

In a large occupational cohort, household exposure to young children was associated with a reduced risk of testing positive for SARS-CoV-2 and COVID-19 requiring hospitalisation (non-significant). Verification of this finding is needed in other populations and settings. Less equivocally, to date, increased household exposure to young children has not been associated with an increased risk of COVID-19, even during periods where schools are open and there is active transmission of SARS-CoV2 in the community. These findings have potential for informing policy on nursery and school closure.

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**1ª associação
LABA/LAMA/ICS**
indicada na ASMA⁴
Dose fixa diária¹

1 vez dia¹

**Zimbus®
breezhaler®**

Indacaterol, glicopirrônio, furoato de mometasona
pó para inalação, cápsulas

**NOVA
ASSOCIAÇÃO
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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 glicopirrônio e furoato de mometasona. Mistura de lactose em pó para inalação, doses únicas, através do dispositivo BREEZHALER® que permite a confirmação de doses¹. *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 IIb, 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 assessment 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 glicopirrônio e 160 microgramas de furoato de mometasona. Cada dose libertada (a dose libertada através do aplicador bucal do inalador) contém 114 microgramas de indacaterol (na forma de acetato), 8 microgramas de brometo de glicopirrônio equivalente a 46 microgramas de glicopirrônio 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 beta₂ 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. **POSOLÓGIA: 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 broncoespasmo, 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. ♦ **Broncoespasmo paradoxal:** Tal como com outra terapêutica inalatória, a administração deste medicamento pode resultar em broncoespasmo 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 cardiovasculares:** Tal como outros medicamentos contendo agonistas adrenérgicos beta₂, 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 tirotóxicos e em doentes com resposta aumentada aos agonistas adrenérgicos beta₂). ♦ **Hipocalcemia:** Os agonistas adrenérgicos beta₂ podem causar uma hipocalcemia significativa em alguns doentes, o que potencialmente pode levar a reações adversas cardiovasculares. Em doentes com asma grave, a hipocalcemia 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 beta₂ e corticosteroides pode produzir um aumento da glicose plasmática. Nos doentes diabéticos, ao iniciar o tratamento, a glicose 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 m²), 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 como 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 coriorretinopatia 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 beta₂, 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 QTc. ♦ **Tratamento hipocalémico:** O tratamento hipocalémico concomitante com derivados da metilxantina, esteróides ou diuréticos não poupadores de potássio pode potenciar o possível efeito hipocalémico dos agonistas adrenérgicos beta₂ e, portanto, a 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 beta₂ de ação prolongada:** A coadministração deste medicamento com outros medicamentos contendo antagonistas muscarínicos de ação prolongada ou agonistas adrenérgicos beta₂ de ação prolongada não foi estudada e não é recomendada. ♦ **Cimetidina ou outros inibidores do transporte de cátiões orgânicos:** Não é esperada qualquer interação medicamento-doença 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 musculoesquelética, espasmos musculares, parestesia. ♦ **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. **Escalação de comparticipação:** Escalão B. **Titular da AIM:** Novartis Europharm Limited. **Representante local:** Laboratório Medifar - Produtos Farmacêuticos, S.A. - Rua Henrique de Paiva Couceiro, N.º 29, Venda Nova, 2700-451 Amadora Informações Essenciais Compatíveis com o Resumo das Características do Medicamento (ZIM_RCM20210422_IEC_v2).

ARTIGO ORIGINAL

Exposição a antibióticos durante a gravidez e asma na criança : um estudo de coorte nacional de nascimentos que investiga o tempo de exposição e o tipo de parto

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RESUMO

Objetivo Investigar se a exposição a antibióticos durante a gravidez está associada à asma infantil e se esta relação foi condicionada pelo tempo de exposição e pelo modo de parto.

Desenho do estudo Um estudo de coorte com recurso a modelos de regressão logística multivariável ajustados para confundidores definidos a priori. Recrutaram-se mulheres grávidas de 1996 a 2002.

Contexto Coorte de Nascimentos Nacional Dinamarquesa.

Doentes Das 96 832 crianças da coorte, foram incluídas 32 651 crianças na população do estudo.

Principal medida de resultados Asma infantil reportada pelos pais de crianças com 11 anos.

Resultados Um total de 5522 (17%) crianças nasceu de mães expostas a antibióticos durante a gravidez. Nas análises ajustadas, as crianças nascidas de mães expostas a antibióticos apresentaram maiores probabilidades de asma (OR 1,14, 95% IC 1,05 a 1,24). Não se verificou uma associação com a exposição a antibióticos no primeiro trimestre (OR 1,02, 95% IC 0,83 a 1,26), mas foram observadas maiores probabilidades para exposição a antibióticos entre o segundo e o terceiro trimestres (OR 1,17, 95% IC 1,06 a 1,28) em comparação com as crianças não expostas a antibióticos. A associação global entre antibióticos durante a gravidez e a asma infantil apenas foi observada em crianças nascidas por parto vaginal (OR 1,17, 95% IC 1,07 a 1,28), mas não em crianças nascidas por cesariana (cesariana planeada: OR 0,95, 95% IC 0,66 a 1,37; cesariana de emergência: OR 0,96, 95% IC 0,73 a 1,28). Nas crianças nascidas por parto vaginal expostas a antibióticos, as probabilidades de asma infantil com necessidade de tratamento durante o ano anterior foram 34% mais elevadas (OR 1,34, 95% IC 1,21 a 1,49) em comparação com as crianças nascidas por parto vaginal não expostas a antibióticos.

Conclusões A exposição a antibióticos no período entre o meio e o final da gravidez está associada a maiores probabilidades de asma infantil em crianças nascidas por parto vaginal. O modo de parto poderá alterar a associação.

INTRODUCTION

In Denmark, as elsewhere, antibiotic use in pregnancy is increasing.^{1,2} A large Danish study estimated that 37% of all infants born in 2010 were exposed to systemic antibiotic use during pregnancy.¹ While maternal antibiotics during pregnancy treat and prevent infections in both mother and fetus,³⁻⁵ potential adverse effects are of considerable concern. Studies have reported associations with adverse outcomes in the offspring later in life,⁵⁻¹¹ including asthma.¹²⁻¹⁴

These outcomes have been suggested to be mediated in part by the effects of antibiotics on the maternal microbiome, which largely determines the initial composition of the infant

What is already known on this topic?

- ▶ Antibiotic use during pregnancy has been suggested to increase the risk of asthma in the offspring, but findings remain inconsistent.
- ▶ Previous studies differ significantly in research design, sample size, diagnostic methods, timing of exposure in pregnancy and follow-up period.

What this study adds?

- ▶ Antibiotic exposure in second to third trimester of pregnancy is associated with higher odds of asthma in vaginally born children but not those born by caesarean section.
- ▶ No evidence of association is observed for antibiotic exposure in the first trimester of pregnancy.
- ▶ Mode of delivery appears to modify the association between antibiotic exposure during pregnancy and childhood asthma.

microbiome.¹⁵ This initial infant microbiome contributes to immune development and is increasingly recognised as a predictor of disease risk in the offspring,¹⁶ but the specific mechanisms underlying prenatal antibiotics and long-term effects on immune-related child health outcomes remains uncertain. Mode of delivery may also determine subsequent immune ontogeny in the offspring, as caesarean section born infants acquire their initial microbiome from maternal skin and the immediate environment, rather than the enteric maternally derived microbiome transferred to vaginally born infants.^{17,18}

The putative association between antibiotic exposure in pregnancy and childhood asthma has been supported by most^{12,14,19-23} but not all studies.^{13,24-27} The inconsistency in findings may reflect differences in sample size, study design, identified confounders, exposure/outcome definitions, timing of exposure and follow-up period. We investigated whether antibiotic exposure during pregnancy was associated with childhood asthma and whether this was conditional on timing of exposure and mode of delivery. We used detailed data on a large population-based cohort to address some of the previous methodological shortcomings.²⁸

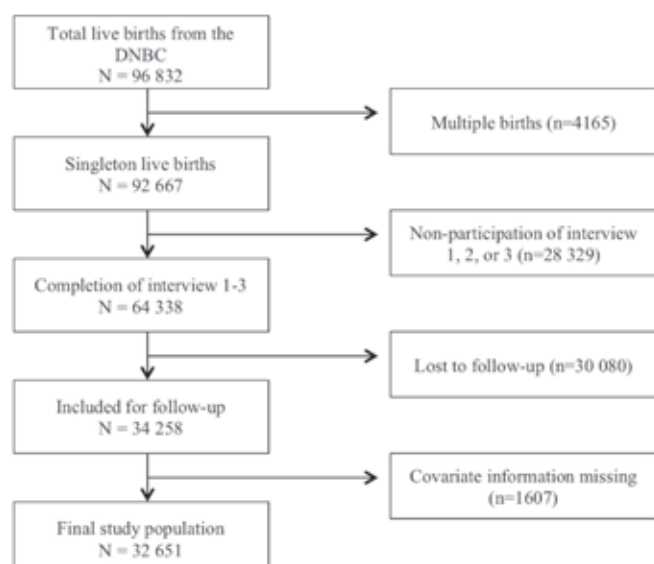


Figure 1 Flow chart of the study population. DNBC, Danish National Birth Cohort.

MATERIALS AND METHODOLOGY

Study design and data source

Participants were from the Danish National Birth Cohort (DNBC), a longitudinal birth cohort established in 1996 to explore health impacts of prenatal and early life exposures.²⁹ Pregnant women were recruited to the DNBC by their general practitioner (GP) at the first antenatal visit at gestational week 6–10. Approximately 60% of all Danish GPs participated in the recruitment between 1996 and 2002, and approximately 30% of all Danish pregnant women were included at the time.³⁰

Information about maternal characteristics, prenatal exposures and child health was obtained by telephone and online questionnaires. Telephone interviews were performed at three time points: twice during pregnancy (16 and 30 weeks' gestation) and once postnatally with retrospective pregnancy information (6 months postpartum). Follow-up information at 11 years was obtained through online questionnaires completed by mothers.²⁹

Study population

The sample population was 96 832 live births from the DNBC. We excluded multiple births (n=4165) and pregnancies where mothers did not complete all baseline interviews (n=64 338). A total of 34 258 (37% of singleton live births) mothers completed the follow-up questionnaire. The final study population included 32 651 singleton children with complete data on covariates (figure 1).

Antibiotic exposure during pregnancy

Information on antibiotic exposure during pregnancy was obtained at second and third interview covering before and after 30 weeks' gestation, respectively ('Have you taken any medication against infection or inflammation during pregnancy? E.g. penicillin, sulfa drug, other antibiotics or medicine against fungus?'). Antifungal medication was coded separately. Information on specific gestational week of antibiotic exposure was recorded. Pregnant women were classified as exposed if they indicated taking antibiotics at any time during pregnancy. To investigate potential impact of timing of exposure and to account for potential latency in antibiotic induced changes to the maternal microbiome, antibiotic use was analysed as early pregnancy (during first trimester), mid-to-late pregnancy (during second to third trimester) and by specific trimester. Pregnant women reporting antibiotics in more than one exposure period in pregnancy were excluded. Pregnant women were classified as unexposed if they had not reported any antibiotics during pregnancy.

Childhood asthma

Information on childhood asthma was obtained from a questionnaire completed by mothers when the child was 11 years old

('Has your child ever had asthma?'). Child's age at the most recent asthma exacerbation and whether they received antiasthma medication in the preceding year was recorded. In sensitivity analyses, two supplementary asthma definitions were included. To differentiate asthma from other respiratory diseases in early childhood with similar symptoms, asthma was defined as 'latest asthma attack at age 5 years or later', and to investigate severity, asthma was defined as 'asthma medication received in the past year'.

Covariates

Potential confounders were identified a priori with data from the DNBC: maternal smoking in pregnancy (yes or no); prepregnancy maternal body mass index (BMI; <18.5, 18.5–24.9, 25–30 and >30 kg/m²); maternal history of asthma (yes or no); paternal history of asthma (yes or no); parity (0, 1 and >1); and socio-occupational status (high, middle and low).³¹ Additional information on sex (male or female), maternal age at delivery (<20, 20–24, 25–29, 30–34 and >34 years), gestational age (<34, 34–36, 37–42 and >42 completed weeks gestation) and mode of delivery (vaginal, planned caesarean section and emergency caesarean section) was obtained from the Danish Medical Birth Registry.³²

Statistical analyses

Descriptive analyses identified characteristics of the study population. Pearson's χ^2 test compared exposed and unexposed children. Multivariable logistic regression models estimated associations between antibiotic exposure during pregnancy and childhood asthma, including timing of exposure. To assess the impact of gestational age and mode of delivery on the overall association, the main analyses were run in three models with and without further adjustments for gestational age and/or mode of delivery. Stratified analyses and effect modifier analyses evaluated possible effect modification by mode of delivery. Sensitivity analyses explored the effect of different asthma definitions. All associations were estimated using Stata V.13.1.

Ethics statements

Written informed consent was obtained from all DNBC participants prior to data collection. Children born into the cohort participated on their mother's consent until age 18 years. Participants could withdraw their data from the cohort at any time (currently <20 mothers and children).²⁹

RESULTS

Of the 32 651 studied children, 5522 (17%) were born to mothers who reported antibiotic use during pregnancy (table 1). Compared with unexposed mothers, mothers exposed to antibiotics during pregnancy reported more maternal asthma, smoking during pregnancy, overweight/obesity, were less likely to be primigravidae, but were similar age. During follow-up, 4238 (13%) children had reported asthma. Of these, 804 (15%) had mothers who were exposed to antibiotics during pregnancy. A total of 4568 (14%) children were delivered by caesarean section.

Overall, antibiotic exposure during pregnancy was associated with higher odds of childhood asthma after adjusting for confounders (model 1: OR 1.14, 95% CI 1.05 to 1.24) (table 2). Results were similar with additional adjustment for gestational age and mode of delivery (model 3: OR 1.13, 95% CI 1.04 to 1.23). The overall absolute risk difference was 1.9% and number needed to harm (NNH) was 53. The higher odds of childhood asthma were only observed for antibiotic exposure in second to third trimester (OR 1.17, 95% CI 1.06 to 1.28), compared with no exposure. This association persisted in the trimester-specific analysis although with wider CIs (second trimester: OR 1.12, 95% CI 0.98 to 1.28; third trimester: OR 1.12, 95% CI 0.98 to 1.28). No association was observed for exposure in the first trimester (OR 1.02, 95% CI 0.83 to 1.26).

The higher odds of asthma after antibiotic exposure was observed in vaginally born children (OR 1.17, 95% CI 1.07 to 1.28) but not in those born by caesarean section (planned: OR 0.95, 95% CI 0.66 to 1.37; emergency: OR 0.96, 95% CI 0.73 to

Table 1 Characteristics of the study population

	Total n (%)	Antibiotic exposure during pregnancy		P value*
		Unexposed n (%)	Exposed n (%)	
All mothers	32 651 (100.0)	27 129 (83.1)	5522 (16.9)	
Sex of child				
Female	16 389 (50.2)	13 634 (50.3)	2755 (49.9)	0.621
Male	16 262 (49.8)	13 495 (49.7)	2767 (50.1)	
Maternal age at birth				
≤19	71 (0.2)	58 (0.2)	13 (0.2)	0.619
20–24	2184 (6.7)	1834 (6.8)	350 (6.3)	
25–29	12 535 (38.4)	10 441 (38.5)	2094 (37.9)	
30–34	12 652 (38.8)	10 490 (38.7)	2162 (39.2)	
≥35	5209 (16.0)	4306 (15.9)	903 (16.4)	
Maternal smoking in pregnancy				
No	25 485 (78.1)	21 250 (78.3)	4235 (76.7)	0.007
Yes	7166 (22.0)	5879 (21.7)	1287 (23.3)	
Maternal BMI before pregnancy				
Underweight (<18.5)	1357 (4.2)	1113 (4.1)	244 (4.4)	<0.001
Normal (18.5–24.9)	23 126 (70.8)	19 363 (71.4)	3763 (68.2)	
Overweight (25–30)	5961 (18.3)	4918 (18.1)	1043 (18.9)	
Obese (>30)	2207 (6.8)	1735 (6.4)	472 (8.6)	
Maternal asthma history				
No	29 984 (91.8)	24 999 (92.2)	4985 (90.3)	<0.001
Yes	2667 (8.2)	2130 (7.9)	537 (9.7)	
Paternal asthma history				
No	30 073 (92.1)	24 999 (92.2)	5074 (91.9)	0.511
Yes	2578 (7.9)	2130 (7.9)	448 (8.1)	
Socio-occupational status†				
High	23 490 (71.9)	19 460 (71.7)	4030 (73.0)	0.020
Middle	8311 (25.5)	6979 (25.7)	1332 (24.1)	
Low	850 (2.6)	690 (2.5)	160 (2.9)	
Parity				
0	15 337 (47.0)	12 961 (47.8)	2376 (43.0)	<0.001
1	12 020 (36.8)	9777 (36.0)	2243 (40.6)	
≥2	5294 (16.2)	4391 (16.2)	903 (16.4)	
Gestational age				
Early preterm birth (<34 weeks)	114 (0.4)	112 (0.4)	29 (0.5)	0.015
Late preterm birth (34–36 weeks)	924 (2.8)	734 (2.7)	190 (3.4)	
Normal birth (37–42 weeks)	31 460 (96.4)	26 177 (96.5)	5283 (95.7)	
Postterm birth (>42 weeks)	126 (0.4)	106 (0.4)	20 (0.4)	
Mode of delivery				
Vaginally born	28 083 (86.0)	23 400 (86.3)	4683 (84.8)	0.018
Planned caesarean section born	1819 (5.6)	1485 (5.5)	334 (6.1)	
Emergency caesarean section born	2749 (8.4)	2244 (8.3)	505 (9.2)	
Childhood asthma				
No	28 413 (87.0)	23 695 (87.3)	4718 (85.4)	<0.001
Yes	4238 (13.0)	3434 (12.7)	804 (14.6)	

*P value calculated using χ^2 test.

†'High'=persons with higher education (4 years beyond high school) or in management; 'middle'=skilled workers and persons with middle-range training; 'low'=unskilled workers and the unemployed.

BMI, body mass index.

1.28) (table 3). Among vaginal births, the absolute risk difference was 2.2% and NNH was 45. There was a weak modifying effect on a multiplicative scale with the joint effect of antibiotic exposure and caesarean section differing from the product of the independent effects (OR 1.09, 95% CI 0.89 to 1.33) (table 4). The

odds of asthma following antibiotics in the first trimester were not evident in vaginally born children nor in caesarean section-born children (OR 1.05, 95% CI 0.84 to 1.31; and OR 0.89, 95% CI 0.52 to 1.52, respectively). For antibiotics in second to third trimester, the odds of asthma were evident in vaginally born

Table 2 Crude and adjusted ORs for childhood asthma according to antibiotic exposure during pregnancy

	Total n (%)	Unadjusted OR OR (95% CI)	Model 1* OR (95% CI)	Model 2† OR (95% CI)	Model 3‡ OR (95% CI)
Antibiotic exposure					
No exposure	27 129 (83.1)	1 (reference)	1 (reference)		
Any exposure	5522 (16.9)	1.18 (1.08 to 1.28)	1.14 (1.05 to 1.24)	1.13 (1.04 to 1.23)	1.13 (1.04 to 1.23)
1. Trimester	853 (2.6)	1.05 (0.86 to 1.29)	1.02 (0.83 to 1.26)	1.02 (0.83 to 1.25)	1.02 (0.83 to 1.25)
2. Trimester	1972 (6.0)	1.14 (1.00 to 1.30)	1.12 (0.98 to 1.28)	1.11 (0.97 to 1.27)	1.11 (0.97 to 1.27)
3. Trimester	2035 (6.2)	1.14 (1.00 to 1.30)	1.12 (0.98 to 1.28)	1.11 (0.98 to 1.27)	1.11 (0.98 to 1.27)
2–3. Trimester	4526 (13.9)	1.20 (1.10 to 1.31)	1.17 (1.06 to 1.28)	1.16 (1.06 to 1.27)	1.16 (1.06 to 1.27)

*Adjusted for sex, maternal age, maternal smoking, maternal BMI, maternal asthma, paternal asthma, socio-occupational status and parity.

†Additionally adjusted for gestational age.

‡Additionally adjusted for gestational age and mode of delivery.

BMI, body mass index.

Table 3 Associations between antibiotics during pregnancy and childhood asthma in all, vaginally born and caesarean section born children

	All children	Vaginally born	Caesarean section born	
	OR (95% CI)	OR (95% CI)	Planned	Emergency
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Antibiotic exposure				
No exposure	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Any exposure	1.14 (1.05 to 1.24)	1.17 (1.07 to 1.28)	0.95 (0.66 to 1.37)	0.96 (0.73 to 1.28)

ORs were adjusted for sex, maternal age, maternal smoking, maternal BMI, maternal asthma, paternal asthma, socio-occupational status and parity.

BMI, body mass index.

children (OR 1.22, 95% CI 1.10 to 1.34), but not in caesarean section born children (OR 0.91, 95% CI 0.71 to 1.16).

Higher odds of childhood asthma were observed regardless of asthma definition (figure 2). The corresponding odds when defining asthma by use of asthma medication in the preceding year were OR 1.30 (95% CI 1.18 to 1.43) and by most recent exacerbation ≥ 5 years were OR 1.21 (95% CI 1.07 to 1.35). Stratified by mode of delivery, significantly higher odds of asthma requiring medication within the preceding year were observed in vaginally born children (OR 1.34, 95% CI 1.21 to 1.49) but essentially unchanged in caesarean section born children (OR 1.06, 95% CI 0.82 to 1.36). Higher odds of asthma attack at age ≥ 5 years were evident in vaginally born children (OR 1.22, 95% CI 1.08 to 1.38) but not in caesarean section born children (OR 1.11, 95% CI 0.83 to 1.49).

DISCUSSION

Antibiotic exposure in mid-to-late pregnancy was consistently associated with childhood asthma in vaginally born children and the association appeared to be modified by mode of delivery. The highest odds were observed in exposed vaginally born children with asthma that required treatment during the preceding year.

Antibiotic exposure closer to delivery (mid-to-late pregnancy), but not in first trimester, was associated with childhood asthma. Exposure in late first trimester may, however, have similar effects to that of early second trimester. We previously reported a similar relationship between timing of antibiotics in pregnancy and risk of infection in

offspring, with a more evident association when exposure was closer to delivery.¹⁶ In other studies, a trimester-specific association has also been suggested, with more significant impact of antibiotics on asthma later in pregnancy.^{12 13 23} In a recent meta-analysis of prenatal antibiotic use in each trimester, a positive association was proposed in all trimesters.²⁷ Our findings suggest that the relationship between antibiotics in pregnancy and childhood asthma may be causal, although confounding by indication remains a possibility.

Our results are in keeping with the hypothesis that effects of antibiotics impact the maternally derived microbiome in vaginally born children and that this may increase the odds of childhood asthma. However, this observational study did not address underlying mechanisms, and this interpretation, while plausible, remains speculative. The effect modifier analysis suggested a modest modifying effect by mode of delivery. The observed association only in vaginally, but not caesarean section, born children may also be attributed in part to a relative small number of exposed women delivering by caesarean section in our study population. Future prospective studies should address specific

Table 4 Effect modifier analysis of antibiotic exposure during pregnancy and mode of delivery on childhood asthma

Antibiotic exposure during pregnancy			
	No exposure	Any exposure	P value for effect modification*
	OR (95% CI)	OR (95% CI)	
Mode of delivery			0.115
Vaginally born	1 (reference)	1.17 (1.07 to 1.28)	
Caesarean section born	1.12 (1.01 to 1.25)	1.09 (0.89 to 1.33)	

ORs were adjusted for sex, maternal age, maternal smoking, maternal BMI, maternal asthma, paternal asthma, socio-occupational status and parity.

*Measure of effect modification on multiplicative scale
BMI, body mass index.

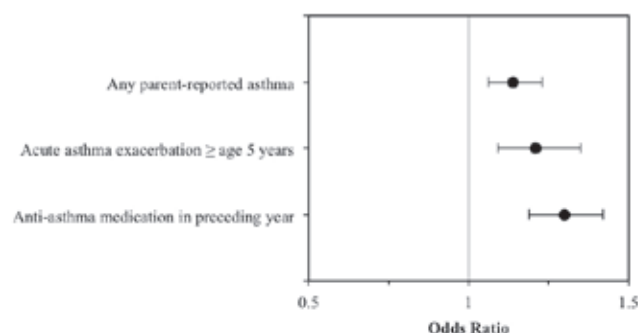


Figure 2 Adjusted ORs for associations between antibiotic exposure during pregnancy and childhood asthma using different asthma definitions. Data points are ORs with 95% CIs adjusted for sex of child, maternal age at delivery, maternal smoking in pregnancy, maternal BMI, maternal asthma, paternal asthma, socio-occupational status and parity. Reference is no exposure of antibiotics during pregnancy. BMI, body mass index.

Table 5 Characteristics of loss to follow-up at 11 years of age

	Total	Status of loss to follow-up		
	Completed baseline	Lost to follow-up	Completed follow-up	P value*
	n (%)	n (%)	n (%)	
Total	64 338 (100.0)	30 080 (46.8)	34 258 (53.3)	
Antibiotic exposure				
No	52 430 (81.5)	24 132 (80.2)	28 298 (82.6)	<0.001
Yes	11 536 (17.9)	5745 (19.1)	5791 (16.9)	
Missing	372 (0.6)	203 (0.7)	169 (0.5)	
Sex of child				
Female	31 501 (49.0)	14 283 (47.5)	17 218 (50.3)	<0.001
Male	32 837 (51.0)	15 797 (52.5)	17 040 (49.7)	
Maternal age at birth				
≤19	276 (0.4)	196 (0.7)	80 (0.2)	<0.001
20–24	5390 (8.4)	3085 (10.3)	2305 (6.7)	
25–29	24 911 (38.7)	11 832 (39.3)	13 079 (38.2)	
30–34	23 989 (37.3)	10 698 (35.6)	13 291 (38.8)	
≥35	772 (15.2)	4269 (14.2)	5503 (16.1)	
Maternal smoking				
No	47 266 (73.5)	20 807 (69.2)	26 459 (77.2)	<0.001
Yes	16 516 (25.7)	8988 (29.9)	7528 (22.0)	
Missing	556 (0.9)	285 (1.0)	271 (0.8)	
Maternal BMI				
Underweight	2723 (4.2)	1311 (4.4)	1412 (4.1)	<0.001
Normal	42 701 (66.4)	18 807 (62.5)	23 894 (69.8)	
Overweight	12 510 (19.4)	6334 (21.1)	6176 (18.0)	
Obese	5378 (8.4)	3103 (10.3)	2275 (6.6)	
Missing	1026 (1.6)	525 (1.8)	501 (1.5)	
Maternal asthma history				
No	58 530 (91.0)	27 120 (90.2)	31 410 (91.7)	<0.001
Yes	5745 (8.9)	2929 (9.7)	2816 (8.2)	
Missing	63 (0.1)	31 (0.1)	32 (0.1)	
Paternal asthma history				
No	58 731 (91.3)	27 376 (91.0)	31 355 (91.5)	0.006
Yes	5189 (8.1)	2481 (8.3)	2708 (7.9)	
Missing	418 (0.7)	223 (0.7)	195 (0.6)	
Socio-occupational status†				
High	43 008 (66.9)	18 488 (61.5)	24 520 (71.6)	<0.001
Middle	18 757 (29.2)	10 003 (33.3)	8754 (25.6)	
Low	2351 (3.7)	1452 (4.8)	899 (2.6)	
Missing	222 (0.4)	137 (0.5)	85 (0.3)	
Parity				
0	29 857 (46.4)	13 712 (45.6)	16 145 (47.1)	<0.001
1	23 791 (37.0)	11 216 (37.3)	12 575 (36.7)	
≥2	10 653 (16.6)	5130 (17.1)	5523 (16.1)	
Missing	37 (0.1)	22 (0.1)	15 (0.1)	
Gestational age				
Early preterm birth (<34 weeks)	493 (0.8)	256 (0.9)	237 (0.7)	1.112
Late preterm birth (34–36 weeks)	1954 (3.0)	935 (3.1)	1019 (3.0)	
Normal birth (37–42 weeks)	61 447 (95.5)	28 687 (96.4)	32 760 (95.6)	
Postterm birth (>42 weeks)	251 (0.4)	119 (0.4)	132 (0.4)	
Missing	193 (0.3)	83 (0.3)	110 (0.3)	
Mode of delivery				
Vaginally born	54 690 (85.0)	25 308 (84.1)	29 382 (85.8)	<0.001
Planned caesarean section born	3783 (5.9)	1859 (6.2)	1924 (5.6)	
Emergency caesarean section born	5865 (9.1)	2913 (9.7)	2952 (8.6)	

*P value calculated using χ^2 test.

†'High'=persons with higher education (4 years beyond high school) or in management; 'middle'=skilled workers and persons with middle-range training; 'low'=unskilled workers and the unemployed.

BMI, body mass index.

underlying mechanisms, particularly the role of the microbiome in immune ontogeny.

Most,^{14 19–23} but not all,^{13 24 25 27} previous studies of antibiotics in pregnancy and childhood asthma report similar results. Discrepancies may reflect methodological heterogeneity, with variations

in follow-up period, study design, sample size and exposure and outcome definitions, particularly in asthma definitions.²⁸ Mulder *et al*¹³ reported no association between the overall use of antibiotics in pregnancy and preschool asthma, performing a prescription-based case–sibling analysis. Örtqvist *et al*²⁴ performed both a

case-sibling analysis and a cohort analysis with no association in the case-sibling analysis, suggesting that associations in the cohort study may reflect confounding by shared familial factors. We attempted to account for shared inherited and environmental factors by adjusting for parental asthma and maternal smoking, but we cannot exclude residual unmeasured confounding, such as paternal/household smoking that we were not able to adjust for.

Several studies have investigated asthma in children <5 years.^{2 12 21 22 24} Among young children, diagnosis of asthma is challenging, and similar symptoms from viral-induced wheeze³³ increase the risk of misclassification. Despite these limitations, Lapin *et al*¹² reported a higher risk of doctor-diagnosed asthma in children ≤3 years of age following prenatal antibiotic exposure. In our study, the sensitivity analysis using alternative definitions to differentiate asthma from early life respiratory infections did not affect the overall findings, and the estimated association was unlikely to reflect misclassification.

We did not adjust for early life antibiotic exposure because childhood antibiotics lie on the causal pathway rather than acting as a confounder. Viral respiratory infections are common in childhood, are often treated (inappropriately) with antibiotics³⁴ and are associated with subsequent asthma.³⁵ Adjusting for antibiotics in early life may underestimate the true effect. For analogous reasons, we did not adjust for breast feeding.

We recognise that maternal infections in pregnancy—rather than or in addition to antibiotics—could affect the association. Studies, including a meta-analysis, indicate that maternal infections in pregnancy increases the risk of childhood asthma^{36 37} but acknowledge the inherent difficulties in differentiating infections from their treatment.

Strengths and limitations

The DNBC enables a large study population with extensive covariate data on child and mother. Unlike most studies, our cohort had a relatively long follow-up, but increased evidence of association in those with more severe asthma (recent medication or exacerbation) gives confidence to the diagnosis of asthma at 11 years. Using interviews to assess antibiotic exposure use may be a more robust measure than prescription-based information, as many pregnant women have poor drug compliance, even after purchasing medication.⁷ A DNBC study estimated low compliance with prescription drugs dispensed during pregnancy, especially those for local or short-term treatments, such as antibiotics.³⁸

We relied solely on maternal reporting for exposure and outcome data. Pregnant women may not recall accurately if and when they took antibiotics; an inherent limitation is the quality of exposure data in the trimester-specific analyses. We attempted to reduce this limitation using repeated standardised questionnaires. Women undergoing caesarean section are given prophylactic antibiotics perinatally, but during most of the study period, the Danish national guidelines were to administer antibiotics after cord clamping³⁹ and therefore would not affect the infant microbiome. Similarly, maternal recall of asthma may be inconsistent, although the diagnosis of childhood asthma at 11 years is usually unequivocal, so misclassification with viral-induced wheeze is less likely than at younger ages.

A total of 30 080 children were excluded because of loss to follow-up. In a separate analysis, characteristics of mothers who were lost to follow-up were fairly similar to mothers in the study population, although mothers lost to follow-up reported higher antibiotic intake, more smoking, were more overweight/obese, younger and more socially disadvantaged (table 5). These differences were relatively small and key covariates were included in the analyses, but generalisability of findings may be limited. In trimester-specific analyses, the generalisability is affected by the exclusion of women reporting antibiotics in more exposure periods.

We were unable to investigate a dose-response relationship of pregnancy antibiotics and childhood asthma as granular data on antibiotic doses were not available from maternal report. Further studies should examine possible dose-response and aim to reproduce our findings in different settings.

CONCLUSIONS

Antibiotic exposure in mid-to-late pregnancy was associated with childhood asthma by 11 years of age in vaginally but not caesarean section born children. Further studies should address the mechanisms underlying this epidemiological observation to identify possible interventions. The profligate use of antibiotics in pregnancy should be balanced against the increasing evidence on adverse long-term health outcomes in the offspring, as well as broader concerns regarding antimicrobial resistance.

Contributors CSU, LHP and BHB designed the study. CSU performed the analyses of the data. All authors contributed to the writing of the paper. BHB supervised the project.

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Patient consent for publication Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available. The data from the study are not publicly available due to considerations for privacy and anonymity of the participants. The data were retrieved from the DNBC and were used under licence for the current study. All requests for data from the DNBC must include a research protocol to be submitted to the Danish Data Protection Agency (Datatilsynet).

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ARTIGO ORIGINAL

O tempo de exposição à *Mycobacterium tuberculosis* explica a variação da eficácia da BCG: uma revisão sistemática e meta-análise

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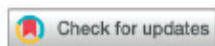
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RESUMO

Racional A heterogeneidade na eficácia observada em estudos de vacinação com BCG não é totalmente explicada pelas hipóteses atualmente aceites, tais como o gradiente latitudinal na exposição a micobactérias não tuberculosas.

Métodos Atualizámos revisões sistemáticas anteriores sobre a eficácia da vacinação com BCG até 31 de dezembro de 2020. Utilizámos uma estratégia de pesquisa e critérios de inclusão/exclusão idênticos a estas revisões anteriores, mas reclassificámos vários estudos, desenvolvemos um sistema de classificação alternativo e considerámos a demografia do estudo, a abordagem diagnóstica e o contexto epidemiológico relacionado com a tuberculose (TB).

Principais resultados Dos 21 ensaios incluídos, aqueles que recrutaram recém-nascidos e crianças com menos de 5 anos foram consistentes na demonstração de uma proteção significativa contra a tuberculose durante vários anos. Os ensaios em cenários de carga elevada com seguimento mais curto também demonstraram uma proteção significativa, tal como a maioria dos ensaios em cenários de diminuição da carga com acompanhamento mais longo. Contudo, os poucos ensaios realizados em cenários de carga elevada com seguimento mais longo não demonstraram qualquer proteção e, por vezes, apresentaram taxas de casos mais elevadas nos vacinados comparativamente aos controlos no período de seguimento posterior.

Conclusões A hipótese explicativa mais plausível para estes resultados é que a BCG protege contra a tuberculose que resulta da exposição pouco tempo depois da vacinação. No entanto, não encontramos provas de proteção quando a exposição ocorre mais tarde após a vacinação, o que seria de maior importância em ensaios com cenários de carga elevada com um acompanhamento mais longo. Em cenários de diminuição da carga, a maior parte da exposição ocorre pouco depois da vacinação e a proteção sustentada observada durante muitos anos depois representa uma proteção contínua contra esta exposição inicial. Contrariamente, em cenários de transmissão intensa e contínua, a proteção inicial diminui subsequentemente com a exposição repetida à *Mycobacterium tuberculosis* ou a outros agentes patogénicos.

INTRODUCTION

Tuberculosis (TB) is the world's leading infectious disease killer,¹ and BCG is the only approved vaccine for its control. Global BCG coverage was 88% in 2019, close to the highest coverage of any vaccination.² The substantial heterogeneity in the efficacy of BCG vaccination between studies has long been recognised, with study-level variables such as age, latitude and BCG strain able to explain some of this variation in meta-analyses unrestricted by age.^{3–5}

Past attempts to understand this heterogeneity have often started from the assumption that protection wanes with time from vaccination.^{6–7} However, time since vaccination parallels immunological maturation and changing TB phenotype,^{8–10} which may lead to confounding. While retrospective national health data have shown

Key messages

What is the key question?

► What is the reason for the extreme variation in efficacy estimates between trials of BCG vaccination?

What is the bottom line?

► Variation in background intensity of *Mycobacterium tuberculosis* transmission is able to explain the considerable heterogeneity in the results of trials of BCG vaccination, with sustained protection more evident in settings of declining transmission.

Why read on?

► We propose a new framework for understanding the substantial variation in BCG vaccination trial results, with profound implications for understanding BCG's effects on the global tuberculosis epidemic.

that vaccine effectiveness can be sustained for >15 years,^{11–12} multiple observational studies in low-burden settings have found that past history of BCG vaccination significantly increases subsequent disease risk in TB contacts.^{13–14}

These observations suggest that intensity or timing of *M. tuberculosis* (Mtb) exposure relative to age and time since vaccination may be important in determining vaccine effectiveness. However, no coherent theoretical framework has been proposed to explain the diversity of results and the adverse effects of BCG sometimes observed. Because of the markedly different disease phenotypes and onset timing relative to age of exposure,^{14–15} we reviewed evidence for the efficacy of BCG vaccination with a focus on TB-related epidemiology, diagnostic approach, time from vaccination and age.

METHODS

Reference management

We performed a systematic review of studies of the effect of BCG vaccination on TB disease, including subcategories of TB disease, with search strategy and inclusion/exclusion criteria identical to Abubakar *et al*⁵ and consistent with our PROSPERO-registered protocol (CRD42019119676), but with search dates extended to 31 December 2020 (section 2, online supplemental appendix). Details of our search strategy are presented in section 13 of online supplemental appendix. We focused on clinical trials as the highest form of evidence, but also reviewed cohort studies as a secondary level of evidence, as presented in the study profile (figure 1). We considered studies comparing participants receiving their first BCG vaccination against unvaccinated controls, excluding

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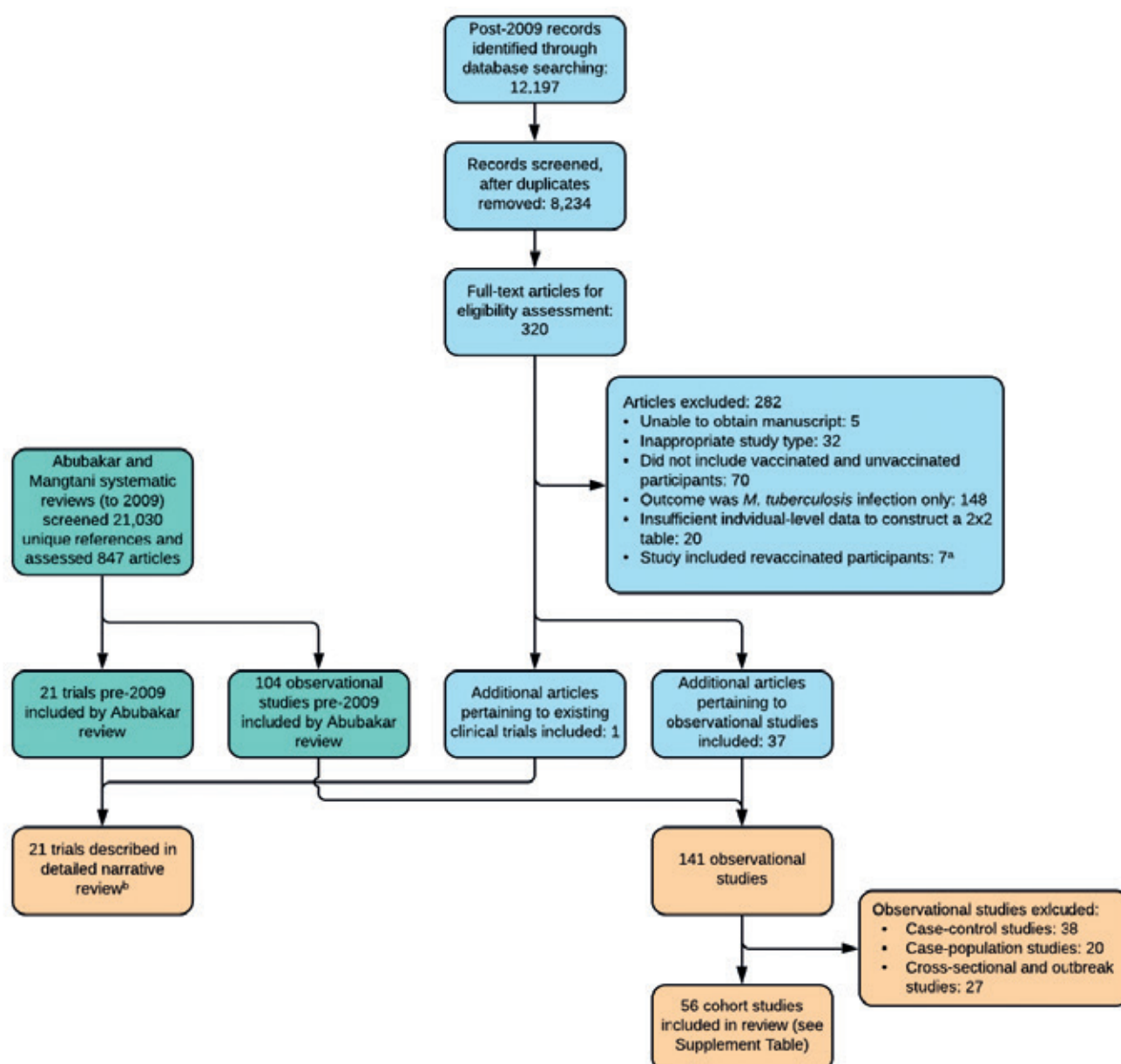


Figure 1 Modified PRISMA flow diagram. ^aIncludes two reports of one revaccination trial which observed zero cases of TB during follow-up. ^bTrials in Chicago medical students, Chicago nursing students and New York infants were not included in Mangtani review. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; TB, tuberculosis.

trials of BCG revaccination, consistent with previous reviews. No further eligible trials were identified that had not been included in the earlier review, with the recent trial of placebo, H4:IC31 vaccine and BCG (which observed no TB cases) excluded as a revaccination trial.¹⁶ All data extracted from studies published from 2009 to 2020 were reviewed by two authors.

Data extraction

We describe all trials in detail in our extended narrative review (sections 3–11, online supplemental appendix). Unlike previous reviews,^{4,5,17} we considered how age-specific reactivation profiles may be influenced by the background intensity of *Mtb* transmission and formulated a new classification for included trials and conceptual framework for integrating their results. Specifically, we considered participant age, local background TB burden and duration of follow-up as the main factors in classifying included trials. Trials not exclusively recruiting neonates have previously been grouped according to study-level factors, including: stringency of latent TB infection (LTBI) testing, as either single or multiple tests; and age group, as either school aged or other age. However, previous reviews classified several studies as ‘other age’, even though most participants were children, which we believe is misleading.^{4,5} We also identified

errors in the previous reviews (section 2.3, online supplemental appendix), including two major trials incorrectly assigned according to the authors’ classification system, with the differences between our results and those of the previous reviews confirmed by three authors blinded to each other’s assessment (JMT, AK and RR).

Because the Chengalpattu trial was the largest ever trial of BCG vaccination, was performed in a high-burden setting and was relatively recent, we also obtained unpublished estimates from this study disaggregated by age and time from vaccination. To illustrate the interacting effects of age and time from vaccination, we extracted data from reports of trials for which data could be disaggregated by both age at TB diagnosis and time from vaccination, in combination with the previously unpublished data from the Chengalpattu trial.

Data synthesis and analysis

We used a hierarchical approach to estimating effect sizes, given the diverse approaches to presenting outcomes. Where possible we estimated effect sizes from information on person-years of follow-up and number of cases occurring in the vaccinated and unvaccinated groups. If this was unavailable, we estimated follow-up periods from the information provided (section 2.2, online supplemental appendix).

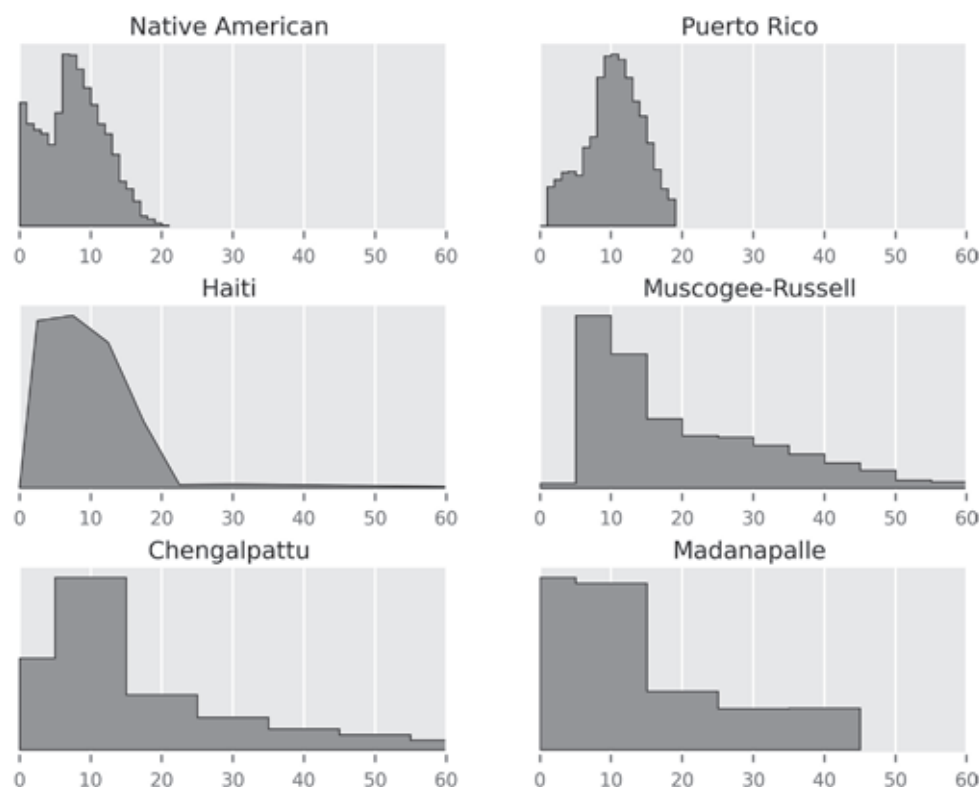


Figure 2 Age distribution (in years) of participants in studies for which these data were provided. Studies with very narrow age inclusion criteria not presented (ie, five neonatal trials and English cities trial of children aged 14–15½ years). All age distributions normalised to the same maximum vertical height.

In our classification, we first grouped all trials in neonates and young children into a single category. Next, we divided the remaining trials into those recruiting predominantly older children and adolescents or those recruiting predominantly adults, and considered the studies with follow-up periods of no more than 5 years separately from those with longer follow-up. Of the remaining trials with longer follow-up, we classified according to whether transmission remained substantial or declined to low levels during the follow-up period. This required some subjective judgement, given that burden indicators were invariably not reported at the exact level that the study was undertaken. However, there were important differences between these studies with regard to TB burden. For the paediatric studies, we considered that burden was likely to have been higher in Puerto Rico by the end of the 1960s (around 12 deaths per 100 000 per year)¹⁸ than it was in the UK in the early 1970s (<2 deaths per 100 000 per year), the USA in the late 1960s (2–3 deaths per 100 000 per year) and the USA in the late 1990s (<0.5 deaths per 100 000 per year). For the adult studies, burden was likely to have been considerably lower in the general population in 1970 than in psychiatric inpatient care in 1960 in the USA, and while India had substantial TB transmission throughout the duration of the Chengalpattu trial, mortality fell approximately ten-fold in Madanapalle due to the highly effective active case finding campaign linked to this BCG trial.¹⁹

Meta-analysis was performed under our new classification with Stata V.16.1. The forest plot was generated using the random effects model of DerSimonian and Laird with the Mantel-Haenszel assessment of heterogeneity from the metan package. Pooled effect size estimates and confidence limits are also presented using REML with Knapp-Hartung adjustment provided by Stata's meta function.

Stata code for meta-analysis and the data and Python 3.6 code for generating figures 2 and 3 are available at https://github.com/jtrauer/bcg_tb_context_review.

RESULTS

Included studies are described in our narrative review (sections 3–11, online supplemental appendix) and summarised as follows and in table 1. Assessment of study quality according to standard criteria for the assessment of clinical trials has been undertaken in previous systematic reviews (online supplemental appendix),⁵ and our assessments did not deviate from these findings.

Neonatal vaccination trials

Of 21 included trials we identified, six recruited neonates only.^{20–25} A small minority of the participants in the Saskatchewan native infants and New York infants trials received oral vaccination, but most infants in these trials received parenteral vaccination. The trial in New York infants reported on TB-related mortality but not TB incidence, finding eight deaths in each of the vaccinated and control groups during its alternate assignment period.²⁶ Although the trial undertaken in the lowest resource and likely highest transmission setting reported the lowest efficacy, estimates of protection were homogeneous, with most trials consistent with the pooled estimate of protection for trials in neonates and young children. Follow-up duration varied, although none followed participants into or beyond adolescence in a setting of continued intense transmission.

Trials recruiting young children

Although the Agra trial was previously included with school-aged vaccination and is only reported very briefly, participants were up to 5 years of age at entry.²⁷ The efficacy in this trial was 60% (95% CI 17% to 81%) protection, consistent with estimates for neonates. The other trial recruiting the youngest children (previously classified as school aged) observed three cases of TB, all among control participants, also consistent with high childhood efficacy.²⁸

Reclassification of trials not restricted to young children

The categorisation of these studies as 'school' or 'other' age fails to convey that many studies previously categorised as 'other' age predominantly recruited children. This often occurred because of an expansive population age pyramid and/or inclusion criteria that typically included negative LTBI testing, the prevalence of which declines with age, particularly in high-burden settings. A prime example is the population-wide Haiti trial, in which tuberculin skin test (TST)-negativity in adults was so rare that the protocol was modified to exclude participants aged over 20, resulting in a predominantly paediatric cohort (figure 2).²⁹

Trials recruiting broadly across paediatric age ranges

Three trials recruited across most or all paediatric age ranges to approximately 20 years.^{29–31} The Haitian trial observed participants for 3 years, with one case occurring in the vaccinated and five in the

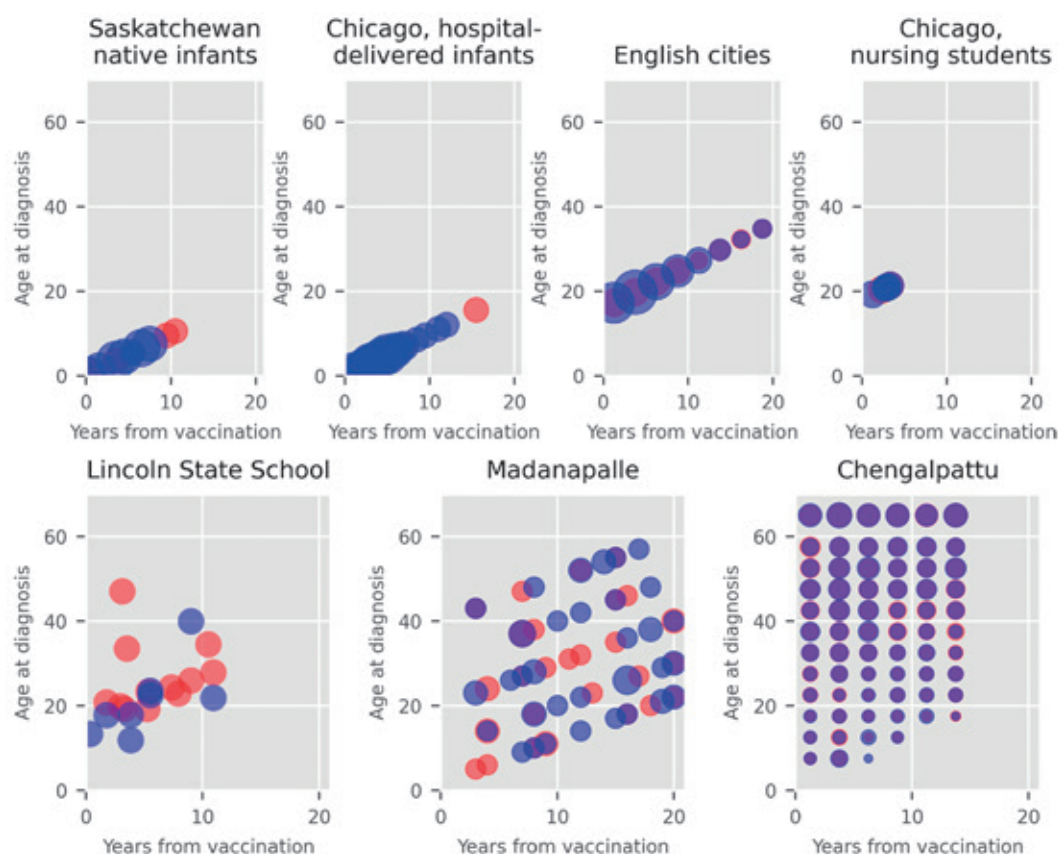


Figure 3 Distribution of cases of active TB occurring in the vaccinated (red) and unvaccinated (blue) populations in which timing of cases by age can be determined to within a 5-year interval. The area of each marker is set proportional to the number of cases occurring within a certain time/age interval and then linearly scaled by an arbitrary value for visual effect. First six studies assigned to vaccinated and control in a 1:1 ratio, while Chengalpattu assigned in ratio of 2 BCG: 1 control, with the size of the vaccinated circles halved to compensate for this effect. Madanapalle panel presents results for bacteriologically-confirmed cases only. First panel data obtained from table IV of Ferguson 1949,²⁰ second panel data obtained from figure 1 of Rosenthal 1961,²³ third panel data obtained from table 3 of MRC 1972,⁵⁴ fourth panel data obtained from table 3 of Rosenthal 1963,³⁷ fifth panel data obtained from tables 1 and 2 of Bettag 1964,⁴⁰ sixth panel data obtained from table 5 of Frimodt-Moller 1973,⁹ seventh panel presents previously unpublished data. TB, tuberculosis.

controls, suggesting good short-term protection in a high transmission setting.²⁹ The large Puerto Rico trial (previously misclassified as non-stringent TST) was undertaken in a setting of rapidly declining but substantial burden and showed modest efficacy.^{31–32} The trial in native Americans was undertaken in a setting in which rates of disease rapidly declined to very low levels throughout most of follow-up, with sustained efficacy for >60 years.^{21–30–33}

Paediatric/adolescent trials

The trial in Georgia schools recruited ages 6–17, with the small number of cases that occurred all accruing after 6 years of follow-up.^{32–34} The large MRC-funded trial was undertaken in school children in large English cities and found high efficacy in a setting of rapidly declining burden (figure 3).^{35–36}

Trials in young adults with shorter follow-up

Three trials from Chicago of participants at high risk because of occupational or residential exposure risk were included in this category, along with one trial of participants at high occupational risk from a high-burden setting. The trial of mental health patients observed only one case of 'bilateral minimal arrested TB' in 35 participants, providing little information. Two reports from Chicago describe BCG vaccination trials in students of nursing and medicine followed for the duration of their studies. Both were small and excluded from previous reviews for methodological limitations, with only 11 cases occurring across both trials.^{4–37–38} The trial in South African mine workers was also excluded from past reviews for including some participants who were TST-positive at baseline,⁶ but found fewer cases in the vaccinated over 3.6 years of follow-up.³⁹

Trials recruiting across all ages with extended follow-up

The trial in Muscogee and Russell counties, USA, achieved broad community participation in those aged over five in a setting of low and

declining transmission, finding slightly fewer cases in the vaccinated.^{32–34}

The trial in Lincoln State School describes an adult cohort followed in a high transmission setting and suggested a trend towards more cases in the vaccinated, particularly after 5 years of follow-up (figure 3).⁴⁰

The Chengalpattu trial was the largest BCG vaccination trial ever undertaken and was among the best reported, although it employed one-stage TST screening.⁴¹ It enrolled participants aged 1 month and above, followed for 15 years in a very high transmission setting and found slightly higher TB rates in the BCG vaccinated, although protection was suggested in children.⁴² The Madanapalle trial⁴³ followed participants for 21 years in an initially high transmission setting and focused on the end point of smear-positive TB, which likely explains the low number of paediatric cases (figure 3).⁹ Although previously classified as employing stringent TST testing, one-stage testing with a cut-off of <5 mm was used in around 95% of participants.

Meta-analysis

Heterogeneity was not observed when studies were grouped according to our classification (figure 4). The pooled estimate for the incidence rate ratio of BCG vaccination in trials of neonates and young children was 0.26 (95% CI 0.17 to 0.35). For other paediatric studies in high-burden settings, we refer readers to the descriptions of the original trials. For paediatric studies with longer follow-up in settings of declining burden, the incidence rate ratio was 0.25 (95% CI 0.20 to 0.30). Trials of adults in high-burden settings with short follow-up durations were methodologically heterogeneous, but the pooled estimate suggested benefit, with an incidence rate ratio of 0.59 (95% CI 0.31 to 0.87). Trials of adults with longer follow-up duration were all consistent with a null overall effect, with marginal protection suggested in settings of declining burden and the Chengalpattu trial dominating the effect estimate in high-burden settings.

Table 1 Characteristics of BCG vaccination trials

Setting	Age profile	Recruitment start, recruitment end, follow-up end*	Long-est follow-up (years)	Epidemiological background	Latitude	TST no, dose	TST cut-off (mm)	Follow-up screen-ing	Diagnostic confirmation	TB endpoint (authors' terminology)	Number recruited vaccinated, controls	TB cases in vaccinated, controls	Incidence rate ratio point estimate (confidence intervals)
Saskatchewan native infants, Canada	Neonates	1933, 1945, 1947	14	Rapidly declining following period of very high burden ⁵⁵	50.8 n	None	N/A	X-ray	X-ray and clinical	TB	306, 303	6, 29	0.19 (0.08 to 0.46)
Native infants, USA	Neonates	1938, 1940, 1946	8	Rapidly declining following period of very high burden ³³	48.8 n and 43.2 n	None	N/A	X-ray, TST	Routine diagnosis after active follow-up ceased	Primary TB	123, 139	4, 11	0.41 (0.13 to 1.29)
New York infants, USA	Neonates	1933, 1944, 1944	11	Rapidly declining following period of very high burden ⁵⁶	40.7 n	None (for majority vaccinated before 1 month of age)	N/A	X-ray, TST	Microbiological or autopsy	None	566, 528	Not reported (TB-related deaths: 8, 8)	N/A (TB mortality: 0.93 (0.35 to 2.49))
Chicago hospital-delivered infants, USA	Neonates	1937, 1956, 1956	19	Very high burden, then rapidly declining ⁵⁷	41.9 n	None	N/A	Clinical, TST, X-ray	Clinical, TST, X-ray, autopsy (X-ray sufficient for diagnosis)	TB	5426, 4128	18, 63	0.22 (0.13 to 0.37)
Chicago household contact infants, USA	Neonates	1940, 1955, 1955	15	Very high burden, then rapidly declining ^{23, 57}	41.9 n	None (if mother not the index)	N/A	Clinical, TST, X-ray	Clinical, TST, X-ray, autopsy (X-ray sufficient for diagnosis)	TB	231, 220	3, 11	0.30 (0.08 to 1.07)
Mumbai infants, India	Neonates	1972, 1972, 1975	2.5	Very high burden ²⁵	19.0 n	None	N/A	Clinical, TST	TST sufficient for diagnosis, although most TB diagnoses had X-ray changes	Primary TB	396, 300	22, 27	0.63 (0.36 to 1.11)
Agra preschool children, India	0–5 years	1979, 1979, 1984	5	Very high burden ²⁷	27.2 n	One-stage, 1 TU PPD-RT23	<10	X-ray, sputum examination	Not stated	Radiologically active or probably active TB	1259, 1259	10, 25	0.40 (0.19 to 0.83)
Chicago housing project, USA	0–12 years	1942, 1955, 1955	13	High burden ^{23, 57}	41.8 n	Two-stage, Vollmer then 100 TU OT TST	Not stated	X-ray, TST	Unclear, presumed X-ray and clinical	Active pulmonary TB	947, 944	0, 3	0.17 (0.01 to 3.32)
Jeremie population-wide, Haiti	All ages, see figure 2	1965, 1966, 1969	3	Very high burden ⁵⁸	18.6 n	One-stage, 5 TU PPD-S	<6	X-ray, TST	Microbiological	TB	635, 338	1, 5	0.11 (0.01 to 0.91)
Native Americans in four states, USA	0–19 years, see figure 1	1935, 1938, 1998	63	Low and declining following a period of very high burden ⁵⁹	32.2 n to 58.8 n	Two-stage, 1 then 250 TU PPD	Not stated	X-ray, TST	X-ray during first 12 years, then microbiological, clinical and other tests	Radiological evidence first 11 years, then by case definitions	1551, 1457	84, 280	0.27 (0.21 to 0.34)

Continued

Table 1 Continued

Setting	Age profile	Recruitment start, follow-up end*	Long-est follow-up (years)	Epidemiological background	Latitude	TST no, dose	TST cut-off (mm)	Follow-up screen-ing	Diagnostic confirmation	TB endpoint (authors' terminology)	Number recruited vaccinated, controls	TB cases in vaccinated, controls	Incidence rate ratio point estimate (confidence intervals)
Georgia schools, USA	6–17 years	1947, 1947, 1967	20	Low and declining following a period of high burden ³²	32.5 n	Two-stage, 5 and 100 TU PPD-RT19-20-21	<5	TST	Clinical, microbiological, X-ray, TST	TB	2498, 2341	5, 3	1.56 (0.37 to 6.54)
English cities, UK	14–15½ years	1950, 1952, 1972	20	Low and declining following a period of high burden ³⁴	51.5 n to 53.5 n	Two-stage, 3 and 100 TU OT	<5	Clinical, TST, X-ray	Clinical, microbiological, pathological	TB	13598, 12 867	62, 248	0.23 (0.18 to 0.31)
Puerto Rico children, USA	1–18 years, see figure 2	1949, 1951, 1969	19.8	Declining but substantial following a period of very high burden ³¹	18.4 n	Predominantly (~76%) two-stage†, 1 then 10 TU PPD-RT19-20-21	<6	No active follow-up	Existing surveillance systems	TB	50634, 27 338	186, 141	0.71 (0.57 to 0.89)
Chicago mental health patients, USA	Adults up to 66 years	1943, 1947, 1947	4	High burden ²⁸	41.9 n	Two-stage, 100 TU OT	Not stated	Not stated	Not stated	Pulmonary TB	20, 15	0, 1 (unconfirmed case)	Not estimated
Chicago nursing students, USA	Presumed young adult	1940, 1953, 1956	3	High rates of exposure, higher in vaccinated ³⁸	41.9 n	Two-stage, 2 and 10 TU OT	<6	TST	Clinical, X-ray, other tests (X-ray sufficient for diagnosis)	TB	231, 263	2, 5	0.45 (0.09 to 2.34)
Chicago medical students, USA	20–37 years	1939, 1952, 1964	4	High rates of exposure ³⁸	41.9 n	Two-stage, 2 and either 10 or 100 TU OT	<6	TST	Clinical, X-ray, microbiological	TB	324, 298	0, 3	0.15 (0.01 to 3.00)
Rand Mines, South Africa	30.3±10.3 years†	1965, 1968, 1968	3.6	Very high burden ⁶⁰	26.2 s	None for most of trial, then one-stage for last 8 months	Not stated	X-ray	X-ray	TB	8317, 7997	29, 45	0.62 (0.39 to 0.99)
Muscogee and Russell Counties population-wide, USA	See figure 2	1950, 1950, 1970	20	Low and declining burden	32.5 n	One-stage, 5 TU PPD-RT-19-20-21	<5	None	Existing surveillance systems	TB	16913, 17 854	32, 36	0.94 (0.58 to 1.51)

Continued

Table 1 Continued

Setting	Age profile	Recruitment start, recruitment end, follow-up end*	Long-est follow-up (years)	Epidemiological background	Latitude	TST no, dose	TST cut-off (mm)	Follow-up screen-ing	Diagnostic confirmation	TB endpoint (authors' terminology)	Number recruited vaccinated, controls	TB cases in vaccinated, controls	Incidence rate ratio point estimate (confidence intervals)
Lincoln State School, USA	Not stated†	1947, 1947, 1960	12	Declining but substantial following a period of very high burden ⁴⁰	40.2° N	Two-stage, 10 and 100 TU OT	Not stated	TST, X-ray	Clinical, microbiological, other tests	TB	531, 494	12, 8	1.38 (0.56 to 3.38)
Chengalpattu population-wide, India	See figure 2	1968, 1971, 1987	15	Very high burden ⁶¹	12.7° N	One-stage, 3 TU PPD-S	<8	X-ray, sputum examination	Culture-positive on ≥1 sputum	Culture-confirmed TB	78693, 39 025	380, 180	1.05 (0.88 to 1.25)

*Italised years indicate inferred/assumed dates/follow-up periods.

†Our assessment differs importantly from that of Mangtani *et al.*⁶.

‡TB cases were aged 15–44, such that 'school' does not imply children.

OT, old tuberculin; PPD, purified protein derivative; TB, tuberculosis; TST, tuberculin skin test; TU, tuberculin units.

Cohort studies

Observational studies are summarised in online supplemental appendix table 1.

Study quality

Study quality is discussed with a focus on TB epidemiological context throughout our narrative review (online supplemental appendix). Standard risk of bias assessments were also performed, with all results identical to those of Abubakar *et al.*⁵ (Appendix 4, first table).

DISCUSSION

Few trials of BCG vaccination followed participants for more than 5 years post-vaccination, but of those that did, settings with sustained exposure risk showed lower effectiveness than those in which risk predominantly accrued shortly after vaccination. Therefore, we propose that timing of *Mtb* exposure, rather than timing of TB disease, should be the main consideration in understanding the differences in BCG efficacy between settings (table 2). In settings of rapidly declining TB burden, exposure is less likely further from vaccination and the effect of BCG in preparing the participant's immune system for early exposure is robust. By contrast, in sustained high-burden settings, participants are more likely to be exposed after a longer period from vaccination when BCG efficacy has waned or after re-exposure has occurred. This interpretation implies that BCG is likely to have little effect in settings of persistent exposure.

None of the eight studies of neonates or young children followed a substantial cohort of participants into the high-risk adolescent period in a high-transmission setting, but all were consistent with significant protection in early life. Among paediatric and adolescent trials, two larger studies in settings of declining burden showed substantial protection, and one suggested short-term protection in a high-burden setting. In adults, four high-quality studies with long-term follow-up have been undertaken, with the two performed in high transmission settings suggesting a null or adverse effect,^{40,41} while one in a low transmission setting^{32,34} and one linked to case finding⁴³ suggested modest protection. Methodologically heterogeneous trials with short follow-up of participants were also consistent with short-term protection in adults.

Included trials predominantly considered persons without prior *Mtb* sensitisation; however, the background intensity of *Mtb* transmission remains relevant to the relative importance of early and late reactivation.⁴⁴ In settings of declining burden, TB episodes many years from vaccination are more likely to represent progression of infection acquired shortly following vaccination. Conversely, in high-burden settings, late-presenting episodes may result from later exposure. Under this conceptual framework, trials in both low-burden and high-burden settings will show high initial effectiveness, whereas later protection is dependent on transmission intensity. As late reactivation is commoner in adolescence and adulthood,¹⁴ these effects will be less apparent in studies that do not follow participants passing into adulthood. The recent BCG and H4:IC31 revaccination trial was conducted in adolescents in a high-burden setting. BCG boosting in those previously BCG-vaccinated decreased persistent *Mtb* sensitisation, consistent with short-term protection from infection.¹⁶

A null effect in studies enrolling across a broad range of ages should not necessarily be interpreted as lesser protection in older participants, because such studies may include substantial cohorts of young children and follow-up invariably emphasises the early post-vaccination period. We found marked and significant effects for young children and for the immediate post-vaccination period, which would be expected to favour a protective overall effect in many trials. Therefore, it is plausible that protection remains present in these groups in some trials with longer follow-up and a broad age range of participants, but is offset by adverse effects in adults in the late post-vaccination period. An example is the Chengalpattu trial which reported considerable protection in children aged <15 during the first 12.5 years of follow-up,⁴² but with a marginally deleterious effect in the trial overall. We expected to observe marked protection in children and in the early post-vaccination period with increased rates of TB in the later follow-up period in adults on reanalysis of this trial. These patterns were only observed to a very limited extent in this trial, and although this pattern was suggested in the Lincoln State School, numbers were lower.

Our framework for understanding BCG efficacy is biologically plausible because the most favourable outcome following exposure is stable immune tolerance, and because the immunology of TB differs fundamentally between infants, children and adults.¹⁰ Infants, with reduced capacity of antigen-presenting cells, show higher mortality and more frequent disseminated TB, which is ameliorated by BCG enhancing early *Mtb* containment.⁴⁵ Ages

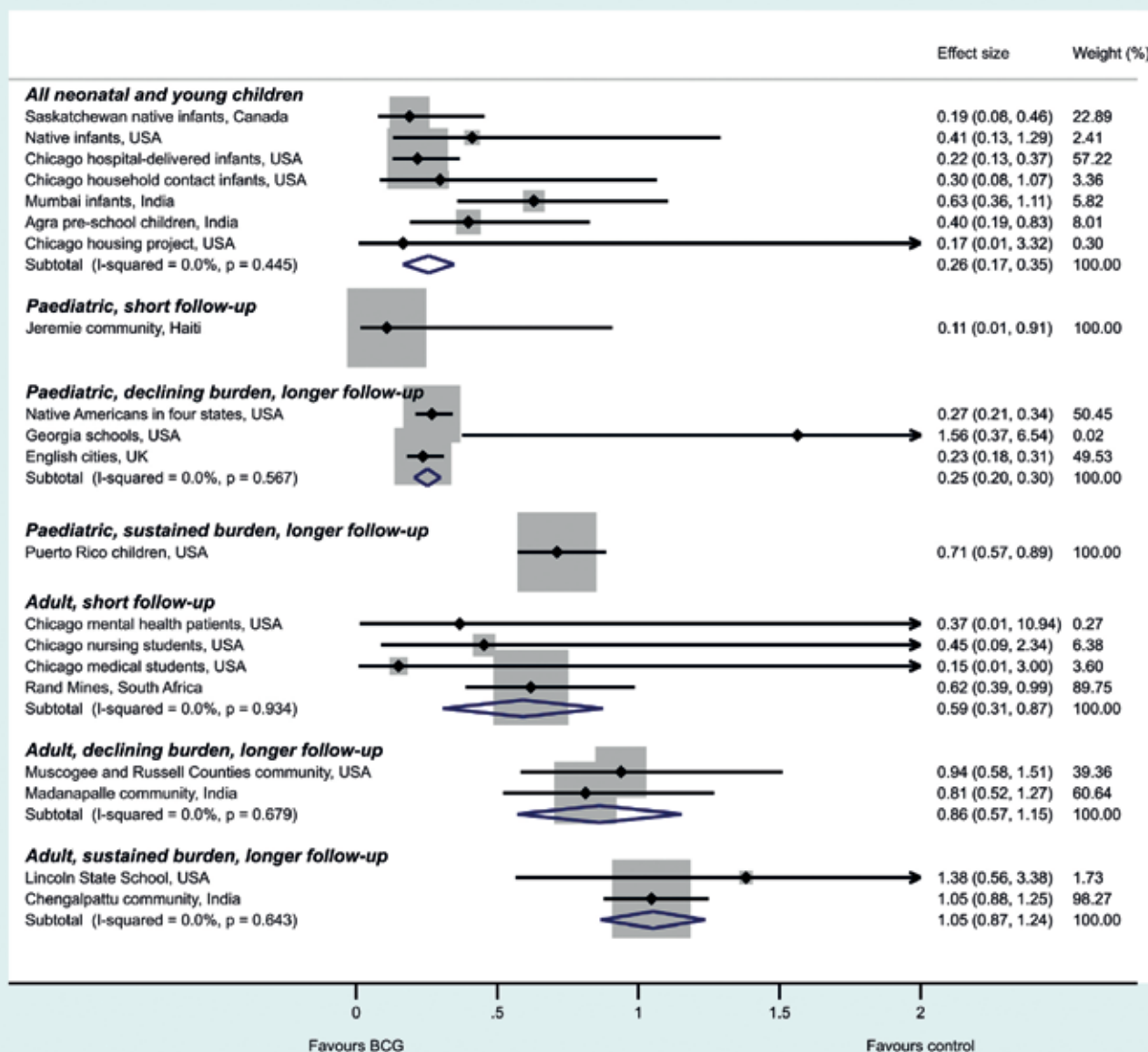


Figure 4 Forest plot of TB incidence rate ratios from trials of BCG vaccination by participant demographics and background epidemiology. Pooled effects are from random effects meta-analysis. Pooled estimates with confidence limits when using a restricted maximum likelihood model with Stata 16.1 and Knapp-Hartung adjustments were: all neonatal and young children: 0.27 (0.15, 0.40); Paediatric/adolescent, declining burden, longer follow-up: 0.25 (0.17, 0.33); adult, short follow-up: 0.59 (0.42, 0.77); adult, declining burden, longer follow-up: 0.86 (0.08, 1.64); adult, high-burden, longer follow-up: 1.05 (0.50, 1.61). New York infants trial not included because only the outcome of TB-related deaths was reported from this trial. TB, tuberculosis.

5–14 years represent an epidemiological paradox of low TB risk despite high Mtb exposure in high-burden settings, during which vaccination may be less important.⁴⁶ However, ‘trained’ immunity, which refers to epigenetic modulation of innate immune cells (monocyte, NK and $\gamma\delta$ T-cells in the case of BCG) may become increasingly important into adulthood, as TB rates increase and the classic pulmonary cavitary disease phenotype emerges.⁴⁷ If by 15–20 years post-vaccination acquired T cell protection wanes, but trained immunity persists, then the host will have lost the beneficial T cell-mediated effects of BCG, but acquired a persistent

hyper-reactive innate response, which is consistent with transcriptomic evidence.⁴⁸ Therefore, because TB disease is immunologically mediated, it is plausible that inducing T cell sensitisation to a broad range of Mtb antigens could protect against the first encounter with the organism during an age of suboptimal immunity, but have a reduced or adverse effect with repeated exposure in adulthood, when the immunological response is vastly different.

Past reviews have proposed that between-trial heterogeneity may be partially explained by latitudinal gradient in non-tuberculous mycobacteria (NTMs) exposure, particularly in studies employing

Table 2 Proposed conceptual framework for understanding the effect of BCG vaccination

Setting	Follow-up period*	Predominant reactivation profile	BCG effectiveness
Declining burden	Early	Early progression from early infection/exposure	High
Declining burden	Late	Late progression from early infection/exposure	High
Sustained high burden	Early	Early progression from early infection/exposure	High
Sustained high burden	Late	Early progression from late infection/exposure	Nil†

*Early: approximately the first 3 to 5 years following vaccination; late: greater than 5 years following vaccination.

†Adverse effect not excluded.

one-stage TST testing.⁴ These arguments require that: (1) NTM sensitisation decreases with latitude, (2) TST positivity detectable only with two-stage testing frequently represents sensitisation to NTMs rather than Mtb, (3) NTM sensitisation confers immunity to Mtb and (4) because of NTM-conferred immunity to Mtb, BCG vaccination boosts immunity to Mtb to a lesser extent in those with NTM sensitisation than those without. Although point (1) is well-established, latitudinal gradient also applies for Mtb. To our knowledge, evidence is conflicting for point (2)³⁸ and derived from animal models for points (3)⁴⁹ and (4).⁵⁰ The greater rates of TB observed in NTM-naïve persons at an equivalent TST response⁵¹ likely reflect a greater probability of Mtb infection. Reanalysis of the Chengalpattu study suggested somewhat lower protection in NTM-sensitised participants,⁵² although the statistical significance of this finding was unclear, while the one trial conducted across sites at multiple latitudes found similar effects by location.³² Although we do not discount the importance of NTM sensitisation, a greater effect of vaccination on clearly TST-negative participants and young children would also support our hypothesis. That is, if the lower efficacy of BCG distant from vaccination is attributable to accumulated exposure to Mtb, then exposure to both Mtb and NTMs could have similar effects in mitigating vaccine efficacy. As such, the recent success of the M72/AS01_E trial in Mtb-exposed, predominantly BCG-vaccinated adults in a high-burden setting supports the need for revaccination with new antigens to mitigate any increased risk from past BCG vaccination.⁵³

We, therefore, believe that our hypothesis is supported by the evidence to a considerably greater extent than previously accepted explanations. However, as is common in empiric research, not every aspect of the analysis is perfectly consistent with the hypothesis. For example, our hypothesis does not explain the adverse effect suggested in the Georgia schools trial, although the confidence intervals were wide due to low case numbers and heterogeneity was not observed in this trial category. We also expected more TB cases in the vaccinated group late from enrolment in the Chengalpattu trial, which was seen to only a minor extent. As previously suggested, this may be explained by high rates of pre-existing Mtb exposure in both the control and vaccinated participants because of one-stage TST testing.

We propose that BCG vaccination protects against early post-vaccination exposure to Mtb, but is ineffective with later exposure, that the results of most or all past trials of BCG vaccination are consistent with this hypothesis and that this framework is more plausible than previously proposed explanations. We believe this explanation is also highly intuitive in retrospect, but may not have been recognised previously because Mtb sensitisation at recruitment has generally been excluded in trial participants based on TST. The absence of an effect late from follow-up in high transmission settings is infrequently observed because it is only seen in studies with long follow-up undertaken in high-burden settings, which are also the most logistically challenging to perform. Given that no clinical trial has found a statistically significant increased risk of disease, any increased rates of TB from late post-vaccination Mtb exposure may not represent an increased lifetime risk in high-burden settings. However, deferring episodes of disease could still have extremely important epidemiological effects, given that paediatric TB more often results in serious sequelae, whereas adult TB is more infectious and critical to perpetuating the epidemic. It is essential that future studies of TB risk and the effect of BCG vaccination present results disaggregated by age and time from exposure to fully elucidate these distinct reactivation profiles.

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Disclaimer The authors acknowledge that the article title for reference Bettag et al, 1964 may cause offence and would like to note that 'mentally retarded' is not an acceptable terminology but is one that was used during the time of the publication.

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Data availability statement Data are available in a public, open access repository. Stata code for meta-analysis, and the data and Python V3.6 code for generating figures 2 and 3 are available at https://github.com/jtrauer/bcg_tb_context_review.

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Associação entre a função pulmonar de crianças em idade escolar e a exposição a curto prazo a poluição e pólenes na atmosfera: A coorte PARIS

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RESUMO

Contexto Os níveis diários de poluição e pólenes na atmosfera poderão afetar a função pulmonar e raramente foram estudadas juntas. Investigamos a exposição a curto prazo aos pólenes e poluição na atmosfera em crianças em idade escolar numa coorte de nascimento de base populacional francesa.

Métodos Este estudo incluiu 1063 crianças da coorte PARIS (do inglês *Pollution and Asthma Risk: an Infant Study*) cuja função pulmonar e medições FeNO foram realizadas aos 8 anos de idade. Os dados da exposição foram recolhidos 4 dias antes da realização dos testes. Estimámos uma concentração de pólenes total diária, os índices de risco alergénico diário para nove táxones de pólen, assim como concentrações diárias de três poluentes atmosféricos (material particulado inferior a 10µm (PM₁₀), dióxido de azoto (NO₂), ozono (O₃)). As crianças com uma exposição a pólenes e poluição na atmosfera semelhante foram agrupadas com recurso a uma análise de *cluster* longitudinal multidimensional. As associações entre os *clusters* de pólenes e poluição na atmosfera e os índices respiratórios (FEV₁, FVC, FeNO) foram estudados usando modelos de regressão logística e linear multivariáveis ajustados para potenciais confundidores.

Resultados Foram identificados quatro *clusters* de exposição: sem pólenes e poluição atmosférica baixa (*Cluster* 1), pólenes de gramíneas (*Cluster* 2), PM₁₀ (*Cluster* 3) e pólenes de bétula/plátano com contagem de pólenes elevada (*Cluster* 4). Comparativamente às crianças no *Cluster* 1, as crianças no *Cluster* 2 apresentaram níveis FEV e FVC baixos e as crianças do *Cluster* 3 apresentaram níveis FeNO mais elevados. Relativamente ao FEV e FVC, as associações aparentaram ser mais fortes nas crianças que padecem atualmente de asma. Análises adicionais sugeriram um efeito conjunto dos pólenes de gramíneas e poluição na atmosfera na função pulmonar.

Conclusão A qualidade biológica e química da ar ambiente diário poderá influenciar negativamente a função pulmonar em crianças.

INTRODUCTION

Lung function may be influenced by current ambient air quality. Most epidemiological studies have focussed on the effects of short-term exposure to ambient air pollutants such as ozone (O₃), nitrogen dioxide (NO₂), particulate matter with aerodynamic diameter <10µm (PM₁₀), <2.5µm (PM_{2.5}) and particularly traffic-related air pollution.¹⁻³ Concerning biological contaminants in ambient air, short-term exposure to pollen significantly increases the risk of allergic and asthmatic symptoms.⁴ Pollen exposure has been little investigated in relation to lung function, especially in population-based studies, although exposure to pollen is a major public health concern, notably in the context of climate change.^{5,6} Epidemiological studies have seldom examined the association of both chemical and biological contaminants on lung function simultaneously, although synergistic effects were suggested in experimental studies.^{7,8}

Key messages

What is the key question?

► Could lung function and FeNO measured in a school-age population-based birth cohort be influenced by both air pollution and pollen levels, in the days leading up to testing?

What is the bottom line?

► We found that children recently exposed to grass pollen had significantly lower FEV₁ and FVC levels, and children recently exposed to PM₁₀ (particulate matter less than 10 µm) had higher FeNO levels, with a possible synergy between grass pollen and air pollution regarding lung function.

Why read on?

► Using an unsupervised approach to identify exposure profiles, we showed that, in a general population setting, children's lung function could be affected by recent air quality in terms of air pollutants and pollens.

Indeed, particulate and chemical pollutants can modulate pollen allergenic potency. Either airborne particles agglomerate onto pollen surfaces in heavy traffic areas⁷ or exposure to different chemical pollutants such as O₃ or NO₂ induces the release of cytoplasmic granules by breaking the outer membrane of the pollen grain.⁸ In their review, Sénéchal *et al*⁹ showed how air pollution can affect the physical, chemical and biological properties of pollen grains. The authors used the concept of 'pollen' to describe the combination of pollen and air pollutants which constitutes a greater risk to respiratory health than pollen or air pollution separately.^{10,11}

Associations between pollen, air pollution and lung function in children have been little studied, and results are heterogeneous and remains uncertain. As part of the BAMSE birth cohort a recent Swedish study suggested a deleterious effect of grass pollen exposure on lung function in children aged 8 after adjusting for air pollution.¹² Previously, Just *et al* found a significant interaction between O₃ and pollen count for asthma attacks in asthmatic children.¹³ During the pollen season, Steerenberg *et al* reported higher levels of fractions of exhaled nitric oxide (FeNO) in pollen-sensitised children than in non-sensitised children, but no additional pro-inflammatory effects of air pollution were observed.¹⁴ Multi-exposure to chemical and biological environmental pollutants can be difficult to

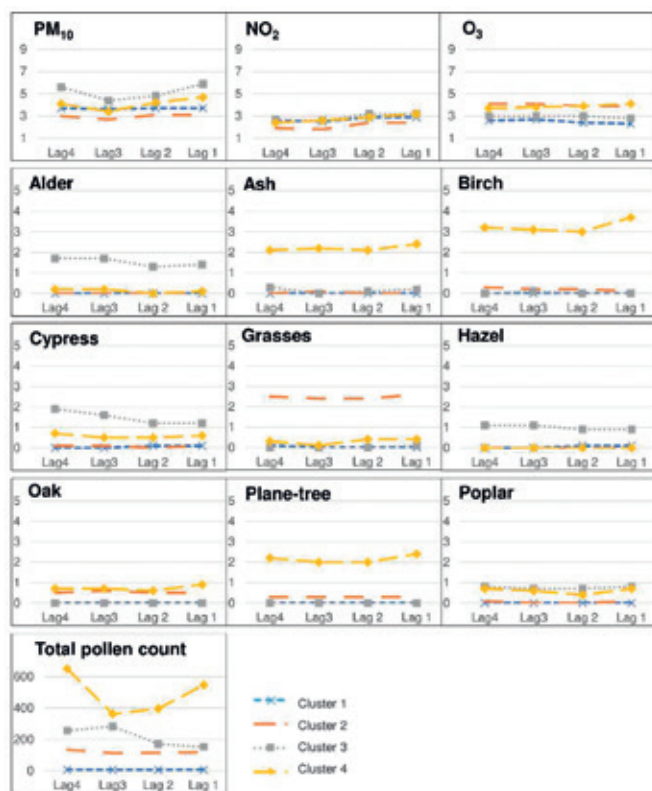


Figure 1 Clusters of recent exposure to air pollution and pollen identified in the 4 days preceding the health check-up at 8 years old in the PARIS birth cohort (n=1063). The x-axis indicates the four time points corresponding to the 4 days (lag4, lag3, lag2, lag1) preceding the health check-up, and the y-axis is the mean value at each time point in each cluster. The variables were coded as follows: PM₁₀, NO₂ and O₃ ATMO subindices from 1 (very good) to 10 (very bad), allergenic risk for each pollen species from 0 (nil) to 5 (very high) and total pollen concentration including 19 pollen taxa in grain/m³. NO₂, nitrogen dioxide; O₃, ozone; PM₁₀, particulate matter with aerodynamic diameter <10 µm.

study because of their correlation. These few studies suggest exposure to certain types of pollen have an effect on certain respiratory/allergic morbidities.

In this context, as part of the PARIS (Pollution and Asthma Risk: an Infant Study) birth cohort follow-up, our aims were (1) to identify groups of children based on their recent exposure to pollen and air pollution before attending a health check-up at 8 years old, and (2) to investigate the short-term association between these groups and lung function, taking into account potential effect modification by current asthma, pollen-induced rhinitis, sex, history of allergy and sensitisation to pollen.

METHODS

Study design and population

The present study was based on the PARIS birth cohort follow-up. Between February 2003 and June 2006, 3840 healthy new-borns were recruited in five Parisian maternity hospitals.¹⁵ Children were followed up using repeated self-administered questionnaires, and two health check-ups were offered at 18 months and 8 years old. Written informed consent was obtained from all parents and children. The present study deals with children who attended the health check-up at 8 years old.

Health data

Health check-ups of the 8 years olds took place in two reference asthma/allergy medical centres in Paris (Necker and Trousseau hospitals) in 2010 to 2015, and included spirometry and FeNO measurements, blood sampling and skin prick test (SPT). In addition, a paediatrician completed a standardised questionnaire including questions about respiratory and allergic symptoms, diagnoses and medications.

Table 1 Characteristics of the 1063 children of the PARIS cohort included in the pollen and air pollution cluster analysis, compared with those not included

	Non included n=2777	Included n=1063	P value*
Sex			0.29
Male	1410 (50.8)	560 (52.7)	
Female	1367 (49.2)	503 (47.3)	
Family socioeconomic status			<0.001
High	1681 (60.5)	713 (67.1)	
Medium	799 (28.8)	281 (26.4)	
Low	297 (10.7)	69 (6.5)	
Older siblings			0.05
None	1604 (57.8)	575 (54.1)	
One	911 (32.8)	365 (34.3)	
Two or more	262 (9.4)	123 (11.6)	
Place of residence at birth			0.34
Paris city	1772 (63.8)	661 (62.2)	
Paris suburbs	1005 (36.2)	402 (37.8)	
Maternal age at birth >35 years	637 (22.9)	308 (29.0)	<0.001
Parental history of asthma	553 (20.0)	211 (19.9)	0.96
Parental history of asthma, allergic rhinitis or eczema	1452 (52.3)	576 (54.2)	0.29
Maternal smoking during pregnancy	331 (11.9)	102 (9.6)	0.04
Exposure to ETS at home at birth	573 (21.5)	224 (21.4)	0.89

All data are n (%). Bold p values were used to highlight significant associations (p<0.05).

*χ² test.

ETS, environmental tobacco smoke.

If applicable, parents were asked to stop any medication inhaled by the child 24 hours before spirometry tests. After checking that the children did not have any respiratory infections during the previous 4 weeks, FeNO measurement was performed using a NIOX MINO electrochemical analyser (Circassia Pharmaceuticals Inc, Chicago, USA). Following this, the best forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) among three reproducible spirometry tests were performed by trained technicians and recorded.¹⁶ From these two indices, the FEV₁/FVC ratio was calculated.

Current asthma at 8 years old was defined according to the definition of the MeDALL (Mechanisms of the Development of ALLergy) consortium, by the presence of two of the following three criteria: ever doctor-diagnosed asthma, use of asthma medication in the last 12 months and wheezing in the last 12 months.

IgE sensitisation to a mixture of inhalant allergens was assessed using ImmunoCAP Phadiatop (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). A positive result (>0.35 kUi/L) led to the measurement of specific IgE towards seven inhalant allergens including birch and timothy pollen.¹⁷ In addition, SPTs were performed including birch, grass and mugwort pollen allergens. We considered children sensitised to pollen if birch or timothy specific IgE concentrations were >0.35 kUi/L or SPT with birch, grass or mugwort was positive (mean weal diameter ≥3 mm).

Environmental exposures

Data on pollen and air pollution were collected for the 4 days prior to the health check-up. Pollen exposure estimates were assessed using data from the French aerobiological monitoring network (RNSA), which is located in central Paris on the roof of Institut Pasteur and was the only monitoring station available at the time of the study. We considered the daily allergenic risk (AR) indices (expressed from 0 to 5) based on the pollen grain count and allergenicity,¹⁸ for each of the following nine taxa: *Alnus*, *Betula*, *Corylus*, *Cupressaceae-Taxaceae*, *Fraxinus*, *Platanus*, *Poaceae*, *Populus*, *Quercus*. In addition, we considered the daily total pollen concentration (expressed in pollen

Table 2 Characteristics of clusters of recent exposure to pollen and air pollution identified at 8 years of age in the PARIS cohort: distribution of exposure levels in the previous 4 days and season of health check-up

	Cluster 1 (‘low exposure’)		Cluster 2 (‘grass pollen’)		Cluster 3 (‘PM ₁₀ ’)		Cluster 4 (‘tree pollen’)	
n (%)	550 (51.7)		327 (30.8)		104 (9.8)		82 (7.7)	
	Median	P25-P75	Median	P25-P75	Median	P25-P75	Median	P25-P75
Average lag1-lag4 daily air pollution ATMO subindices (from 1 to 10)*								
PM ₁₀	4	3–5	3	3–3	6	4–7	4	3–6
NO ₂	3	3–3	2	2–3	3	3–3	3	3–3
O ₃	3	2–3	4	3–5	3	3–3	4	4–4
Average lag1-lag4 daily pollen concentration (in grain/m ³)								
Alder	0	0–0	0	0–0	23.4	16.8–41.1	1.6	0.8–2.5
Ash	0	0–0	0	0–1.0	1.6	0.4–2.8	33.9	26.7–126.6
Birch	0	0–0	0.2	0–1.6	0.2	0–1.0	174.5	36.1–269.1
Cypress	0	0–0	4.9	3.1–7.6	70.6	34.9–131.4	15.2	8.4–32.9
Grasses	0	0–0	19.9	6.8–38.6	0	0–0.2	0.8	0.4–1
Hazel	0	0–0	0	0–0	12.1	4.5–20.5	0	0–0.4
Oak	0	0–0	2.7	0–17.2	0	0–0	2.1	0.6–48.7
Plane-tree	0	0–0	0.2	0–3.7	0	0–0	153.9	1.0–252.3
Poplar	0	0–0	0	0–0.4	5.6	0.8–14.5	6.6	4.3–13.7
Daily total concentration	0	0–1.4	113.5	60.6–176.1	154.4	92.5–250.2	509.5	428.2–666.4
Season of health check-up, n (%)								
Spring	29 (5.3)		233 (71.3)		15 (14.4)		82 (100.0)	
Summer	41 (7.4)		94 (28.7)		0 (0.0)		0 (0.0)	
Autumn	287 (52.2)		0 (0.0)		0 (0.0)		0 (0.0)	
Winter	193 (35.1)		0 (0.0)		89 (85.6)		0 (0.0)	

P25: 25th percentile, P75: 75th percentile.

*1: no or low level of pollution, 10: very high level of pollution.

NO₂, nitrogen dioxide; O₃, ozone; PM₁₀, particulate matter with aerodynamic diameter <10 µm.

grains/m³) based on the following taxa: *Ambrosia*, *Artemisia*, *Alnus*, *Betula*, *Carpinus*, *Castanea*, *Quercus*, *Cupressaceae-Taxaceae*, *Fragaria*, *Poaceae*, *Corylus*, *Olea*, *Rumex*, *Populus*, *Plantaginaceae*, *Platanus*, *Salix*, *Tilia*, *Urticaceae*.

Exposure to air pollution was assessed using an aggregated index created by the air quality monitoring network for the Paris region (AIRPARIF). This ATMO index is based on daily ambient air concentrations of four pollutants subject to regulation (PM₁₀, NO₂, O₃ and sulphur dioxide (SO₂)), which are measured by all background stations located in central Paris and suburbs (n=28). The daily ATMO index representative of both Paris and suburbs is calculated as the highest score among the four ATMO subindices for PM₁₀, NO₂, O₃ and SO₂, expressed from 1 (no or low level of pollution) to 10 (very high level of pollution). The PM₁₀ subindex is based on the average of daily mean concentration while the NO₂, O₃ and SO₂ subindices are based on average of maximum hourly concentrations.¹⁹

In addition, the average ambient temperature and relative humidity were obtained from the French national meteorological service (Météo France) for the Paris-Montsouris station.

Covariates

Information such as sex, maternal smoking during pregnancy, place of residence (Paris, suburbs), and parental history of allergy, occupation and geographical origin was collected at maternity hospital during an interview with the mother. Family socioeconomic status (SES) was based on the highest position among both parents and divided into three categories. Exposure to environmental tobacco smoke (ETS) at home at 8 years old was documented using a parental questionnaire.

Statistical analysis

In order to group children with regards to their pollen and air pollution exposure in the 4 days before spirometry and FeNO testing, we used multidimensional, longitudinal cluster analysis including 3 variables for air pollution (daily PM₁₀, NO₂ and O₃

ATMO subindices) and 10 variables for pollen (daily total pollen count and AR indices for nine types of pollen). All 13 variables were standardised and considered at four time points corresponding to the previous 4 days (lag4, lag3, lag2, lag1). SO₂ was not considered in the analysis because it is no longer a major pollutant in the Paris region. Cluster analysis was performed using a version of K-means clustering adapted to the analysis of joint trajectories.²⁰ The optimal number of clusters was determined based on the Calinski-Harabasz criterion and the clusters considered relevant by experts in aerobiology and pollen monitoring. The analysis was performed on children with data available for ≥2 days for each variable (n=1063) and sensitivity analysis was conducted on children with complete data (n=1055). Each cluster was described by the distribution of average lag1-lag4 for pollen concentration and ATMO subindices.

In addition to the unsupervised analysis described above, and in order to test the possible joint effect of exposure to grass pollen and air pollution on lung function, four groups were created based on the maximum daily ATMO index and grass pollen concentration in the previous 4 days. Thresholds were chosen using a level of 6 for ATMO index (corresponding to poor air quality) and 10 grains/m³ for grass pollen (corresponding to an intermediate AR index).

Associations between clusters of recent exposure or groups of joint exposure and respiratory indices were studied using multivariable, linear regression models for FEV₁ (mL), FVC (mL) and FEV₁/FVC ratio (%), and logistic regression model for FeNO >20 ppb²¹ adjusted for potential confounders identified in the literature and using a directed acyclic graph (DAG).²² All models were adjusted for sex, ethnicity, family SES (based on the highest SES position among parents), age and height at health check-up, exposure to environmental tobacco smoke at home at 8 years old, current asthma and average ambient temperature and relative humidity to the day before the health check-up. FeNO was considered with a threshold as the distribution was not normal. Results were expressed as adjusted regression coefficients (aβ) or adjusted ORs (aORs) with their 95% CIs. When possible, potential effect

Table 3 Associations between clusters of recent exposure to pollen and air pollution and respiratory indices in children aged 8 years in the PARIS birth cohort: multivariable analysis

Exposure clusters	FEV ₁ (mL) n=973	FVC (mL) n=973
	aβ (95% CI)	aβ (95% CI)
Cluster 1	Reference	Reference
Cluster 2	-40.3 (-75.2 to -5.4)	-57.2 (-99.2 to -15.2)
Cluster 3	-13.8 (-55.9 to 28.3)	-31.5 (-82.1 to 19.1)
Cluster 4	-21.5 (-74.3 to 31.3)	-32.7 (-96.2 to 30.8)

Exposure clusters	FEV ₁ /FVC (%) n=973	FeNO>20 ppb n=866
	aβ (95% CI)	n/N aOR (95% CI)
Cluster 1	Reference	84/449 Reference
Cluster 2	0.5 (-0.5 to 1.5)	52/260 0.91 (0.53 to 1.55)
Cluster 3	0.6 (-0.6 to 1.8)	24/84 1.85 (1.01 to 3.37)
Cluster 4	0.3 (-1.2 to 1.9)	15/73 0.83 (0.38 to 1.83)

Cluster 1: 'low exposure', Cluster 2: 'grass pollen exposure', Cluster 3: 'PM₁₀ exposure', Cluster 4: 'tree pollen exposure'.

All models were adjusted for sex, age and height at the health check-up, ethnicity, family socioeconomic status, exposure to environmental tobacco smoke at home at 8 years of age, current asthma and average ambient temperature and relative humidity the day before the health check-up. The estimates in bold differ significantly from the reference ($p < 0.05$). Total N for each model may vary due to missing information.

aβ, adjusted regression coefficient; aOR, adjusted OR; FeNO, fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

modification by current asthma, pollen-induced rhinitis, sex and sensitisation to pollen at 8 years old was explored by testing multiplicative interaction terms using a significance level of 0.10.

Cluster analyses were conducted using R software (V3.5.2, R foundation for Statistical Computing, Vienna, Austria). Regression analyses were performed using Stata/SE 15.1 (StataCorp, College Station, Texas, USA). We followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology recommendations for reporting observational cohort studies.²³

RESULTS

Participants

Compared with children from the original PARIS cohort not included in the present study, children participating ($n=1063$) were more likely to live in a family with high SES, to have older siblings, a

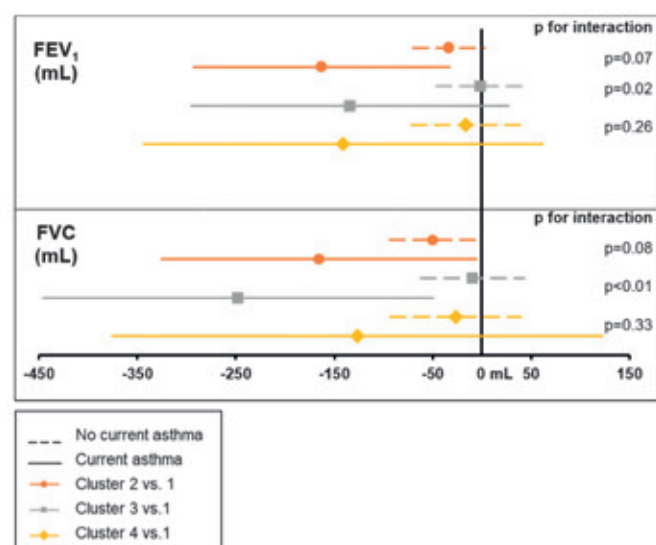


Figure 2 Associations between clusters of recent exposure to air pollution and pollen and respiratory indices at 8 years old according to current asthma status: multivariable analysis in the PARIS birth cohort. Squares represent the adjusted β regression coefficients, and horizontal lines the 95% CIs. Models were adjusted for the same variables as in table 3.

mother aged >35 years at birth and less likely to have a mother who smoked during pregnancy (table 1). However, the participants did not differ regarding sex, place of residence at birth, parental history of asthma or allergy and exposure to ETs at birth (table 1). The selection of study participants is shown in flowchart (online supplemental figure S1). Of the children, 47.3% were women, 8.8% of children suffered from asthma, 6.8% suffered from pollen-induced rhinitis and 18.0% were sensitised to pollen. The description of pollen and air pollution for participants is given in online supplemental table S1.

Clusters of recent exposure to pollen and air pollution

The four-cluster classification provided the second best Calinski-Harabasz criterion (online supplemental table S2) and optimal relevance according to pollen experts, leading to the following clusters (figure 1 and online supplemental figure S2):

- Cluster 1 ('low exposure') showed no pollen exposure and low exposure to air pollution and was considered as reference (51.7% of children).
- Cluster 2 ('grass pollen') was characterised by moderate exposure to grass pollen and low exposure to air pollution (30.8% of children).
- Cluster 3 ('PM₁₀') experienced the highest exposure to PM₁₀ and low exposure to pollen (9.8% of children).
- Cluster 4 ('tree pollen') showed the highest total pollen count, high exposure to birch pollen, moderate exposure to plane-tree pollen and low exposure to other pollens and air pollution (7.7% of children).

These clusters clearly depended on the season of the health check-up (table 2). Indeed, the health check-ups of Cluster 1 were mainly carried out in autumn, Cluster 2 at the end of spring/beginning of summer, Cluster 3 in winter and children from Cluster 4 attended the health check-up at the beginning of spring. The sensitivity analyses performed in children without any missing data led to similar clusters (online supplemental figure S3 and table S3).

Clusters of recent exposure and lung function in children aged 8 years

Associations between the exposure clusters previously identified and respiratory indices are shown in table 3. Lower FEV₁ and FVC values were observed in Clusters 2, 3 and 4 when compared with the reference group. Compared with children in the 'low exposure' cluster, children in the 'grass pollen' cluster had significant lower FEV₁ and FVC (aβ=-40.3 mL, 95% CI: -75.2 to -5.4 and aβ=-57.2 mL, 95% CI: -99.2 to -15.2, respectively). Children in the 'PM₁₀' cluster were more likely to have a FeNO >20 ppb (aOR=1.85, 95% CI: 1.01 to 3.37). No significant association was found in the 'tree pollen' cluster.

Effect modification

Significant interactions between clusters of recent exposure and those who had current asthma were found for FEV₁ and FVC. The deleterious association between the 'grass pollen' and 'PM₁₀' clusters and lung function was stronger in children suffering from current asthma (figure 2).

Among children who had current asthma, compared with children in the 'low exposure' cluster, those in the 'grass pollen' cluster had a significant lower FEV₁ (aβ=-163.0 mL, 95% CI: -292.9 to -33.1) and FVC (aβ=-165.4 mL, 95% CI: -325.1 to -5.6). Those in the 'PM₁₀' cluster had a lower FVC (aβ=-247.8 mL, 95% CI: -445.4 to -50.1). Lung function indices did not differ according to clusters of recent exposure among children who did not have current asthma. No effect modification was found with pollen-induced rhinitis, sex and sensitisation to pollen.

Joint exposure to air pollution and grass pollen and lung function

Half of the children (51%) had low exposure to both grass pollen and air pollution in the previous 4 days before health check-ups (Group 1), 25% had low exposure to grass pollen and high exposure to air pollution (Group 2), 19% had high exposure to grass pollen and low exposure to air pollution (Group 3) and 5% (Group 4) had high exposure to both grass pollen and air pollution.

Table 4 Associations between groups of combined exposure based on maximum daily levels of grass pollen and air pollution exposure in the previous 4 days and respiratory indices in the PARIS birth cohort: multivariable analysis

			FEV ₁ (mL) n=973	FVC (mL) n=973	
	Grass pollen >10 grains/m ³	Poor air quality*	aβ (95% CI)	aβ (95% CI)	
Group 1	No	No	Reference	Reference	
Group 2	No	Yes	7.7 (−20.9 to 36.3)	8.3 (−26.1 to 42.6)	
Group 3	Yes	No	−19.9 (−55.7 to 15.9)	−41.0 (−84.1 to 2.1)	
Group 4	Yes	Yes	−70.2 (−129.7 to −10.7)	−92.5 (−164.1 to −20.9)	

			FEV ₁ /FVC (%) n=973	FeNO>20 ppb n=866	
	Grass pollen >10 grains/m ³	Poor air quality*	aβ (95% CI)	n/N	aOR (95% CI)
Group 1	No	No	Reference	86/446	Reference
Group 2	No	Yes	−0.1 (−1.0 to 0.7)	55/227	1.38 (0.90 to 2.11)
Group 3	Yes	No	0.8 (−0.3 to 1.8)	26/153	0.70 (0.40 to 1.24)
Group 4	Yes	Yes	0.9 (−0.9 to 2.6)	8/40	0.81 (0.33 to 2.03)

All models were adjusted for sex, age and height at the health check-up, ethnicity, family SES, exposure to environmental tobacco smoke at home at 8 years of age, current asthma and average ambient temperature and relative humidity the day before the health check-up. The estimates in bold differ significantly from the reference ($p < 0.05$). Total N for each model may vary due to missing information.

*Poor air quality was defined as an air quality index ATMO ≥ 6 (on a scale of 1 to 10) based on the daily average air concentrations of PM₁₀, NO₂, O₃ and SO₂.

aβ, adjusted regression coefficient; aOR, adjusted OR; FeNO, fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; NO₂, nitrogen dioxide; O₃, ozone; PM₁₀, particulate matter with aerodynamic diameter $< 10 \mu\text{m}$; SO₂, sulphur dioxide.

Associations between these groups and respiratory indices are shown in table 4. Groups 3 and 4 had a lower FEV₁ and FVC. Compared with Group 1, children from Group 4 had a significant lower FEV₁ (aβ=−70.2 mL, 95% CI: −129.7 to −10.7) and FVC (aβ=−92.5 mL, 95% CI: −164.1 to −20.9). Group 3 tended to have a lower FVC (aβ=−41.0 mL, 95% CI: −84.1 to 2.1). No significant association was observed for Groups 2 and 3. No effect modification was found with pollen-induced rhinitis, sex and sensitisation to pollen. Due to small numbers, effect modification by current asthma was not tested.

DISCUSSION

Key results

In the population-based PARIS birth cohort, we applied an unsupervised approach to identify the exposure of school aged children to pollen and air pollution a few days before spirometry and FeNO measurements. We showed that recent exposure to grass pollen with low air pollution levels was associated with lower lung function, compared with children exposed to low air pollution and pollen levels. This effect appeared higher in children with current asthma. In addition, the group with high PM₁₀ levels and low pollen exposure was associated with increased bronchial inflammation as assessed by FeNO. Additional analyses suggested a synergy between exposure to grass pollen and poor air quality regarding lung function indices.

Strengths and limitations

One of the strengths of this study is its reliance on a prospective birth cohort, whose population is well documented from a health, sociodemographic, behavioural and environmental point of view. The objective measurements of lung function were performed during a standardised medical examination in two specialised paediatric pulmonology departments, ensuring the quality of clinical data, thereby reducing measurement and classification bias.

Exposure during the 4 days before the health check-up precisely measured by two French networks specialising in air pollution and pollen monitoring. Other studies have dealt with exposure to pollen using various indicators, mostly surrogate variables such as pollen season^{14 24–27} or pollen concentration for a single type^{12 28 29} or total count.^{13 30} The interest of the indices we used to describe children's exposure to pollen relies on their integrative nature. These indices which combined concentrations (pollen counts) and potential health risk (allergenicity differing according to pollen type) were used for the first time in an epidemiological study on children's lung function. Similarly, the ATMO indices allowed us to assess exposure to air pollution according to pollutant concentration and the potential health risks associated with air quality.

Another strength is the identification of multi-pollutant exposure clusters, jointly considering exposure to air pollution and pollen using a multidimensional classification method. This method better reflects

the reality of multiple exposures and avoids possible correlations between air pollution and pollen exposure, whereas most previous studies of lung function focussed on a single exposure, either to pollen or air pollution.^{25 26 31} Moreover, regression models were adjusted for ambient air temperature and relative humidity, which could be associated with both exposure to pollen/air pollution and lung function indices.³² Meteorological parameters are not always considered as possible confounding factors of the relations between pollution and respiratory health in individual observational studies,^{12 25 26} limiting the interpretation of the results. In addition, our models were not adjusted for season: it was not necessary to do so because season reflects meteorological parameters and respiratory infectious diseases and these variables are controlled in the multivariable models.

As frequently observed in cohort follow-up, our study sample shows an over-representation of high SES families but adjusting for family SES should avoid confounding bias regarding the association between exposure and lung function. Although based on a prospective cohort study, the present research is limited in its cross-sectional approach as it was not possible to study the temporal co-variations between exposure and lung function indices. A panel study or cohort study with repeated measures could explore lung function in relation to daily variation in pollen and air pollution levels. We were not able to investigate the risk of having lung function below a clinical threshold due to the small number of children with such outcomes in our study sample from the general population.

Lastly, pollen exposure was assessed using the only monitoring station thought the children from the PARIS cohort lived in central Paris or its suburbs. In the same way, in the Swedish BAMSE cohort composed of children living in the Stockholm region, pollen exposure was derived from a roof-level urban background monitoring station within central Stockholm.¹² Concerning air pollution exposure, we used air quality indices which were less sophisticated than concentrations measured from the closest background monitoring station but which are relevant in distinguishing days with 'good' and 'poor' air quality. This can lead to non-differential misclassification bias which would underestimate the real associations. However, the use of these indices could allow to include the possible mobility of children within the Paris region in the few days before health check-up.

Interpretation

The present study suggests a deleterious effect of grass pollen on FEV₁ and FVC. In the literature, few studies have examined associations between grass pollen and lung function and results are inconsistent. Two previous studies found no significant association between grass pollen season and FEV₁ in children with asthma²⁷ and in children with seasonal allergic asthma.²⁶ Conversely, in children with seasonal allergic rhinitis, FEV₁ was lower than in the control group during the pollen season (including grass season).²⁵

Our results are consistent with findings from the BAMSE birth cohort,¹² where exposure to grass pollen up to 6 days before lung function testing was associated with a lower FEV₁ in children of 8 years old ($\alpha\beta = -32.4$ mL, 95% CI: -50.6 to -14.2) with an increase in 3 counts/m³. Similar associations were shown with FVC. We did not find any association between grass pollen exposure and FeNO. In allergic children, Steerenberg *et al.*¹⁴ showed significant positive associations between levels of grass pollen in the 4 days before testing and FeNO. Similarly, in asthmatic children, two studies^{26,27} showed an increased level of FeNO during grass pollen season. However, contrary to our study conducted on the general population, these two studies included children more susceptible to suffering adverse health effects from pollen exposure.

In our study, the association between grass pollen exposure and lung function appeared stronger in children with current asthma. No evidence of an effect modification by sensitisation to pollen was found but the number of children sensitised to pollen in the 'grass pollen' cluster was very small. In the BAMSE study, associations between grass pollen and lung function in children were modified by sensitisation to pollen allergens.¹² A recent meta-analysis of panel studies concluded that pollen exposure during the same day increases respiratory symptoms on the same day, as well as specifically upper respiratory and ocular symptoms among allergic and asthmatic participants but there was no detected effect on lung function.⁴

As with the BAMSE study, we did not find any association between tree pollen and children's respiratory indices.¹² This might be explained because children from the 'grass pollen' cluster, who were exposed in late spring-summer, spent more time outside than children from the 'tree pollen' cluster, who were exposed in early spring at the beginning of the pollen season.

In our study, the cluster represented by PM₁₀ exposure was associated with higher levels of FeNO, a marker for airway inflammation. Interestingly, this association was observed in all children and was not modified by sex, sensitisation or current asthma. In the literature, Berhane *et al.* found that short-term increase in PM₁₀ was associated with FeNO independently of asthma and allergy status in a Southern California cohort.³¹ Similarly, Carlsen *et al.*²⁸ found that PM_{coarse} (2.5–10 µm in aerodynamic diameter) was associated with increased FeNO in non-asthmatic children aged 11 years.

Experimental studies showed that particles aggregate on the surface of pollen grains in urban areas.⁷ Moreover, Motta *et al.*⁸ suggested an effect of traffic-related pollutants on pollen cytoplasmic granules (PCG), which contain allergens. After exposure to NO₂ or O₃ the grass pollen grain is damaged and emits PCG. In the present epidemiological study, the issue of the combined effect of air pollution and pollen was not resolved in the cluster analysis. Consequently, we performed additional analyses which showed a joint deleterious effect of poor air quality and grass pollen level ≥ 10 grains/m³ during the previous days on the lung function (FEV₁ and FVC) of school-age children.

Very few epidemiological studies have investigated the combined effect of air pollution and pollen on lung function, especially in children. In these studies, exposure to pollen and pollution is most often accounted for by mutual adjustment in the statistical models,¹² or by testing the interaction between two exposure variables.^{13,14,28–30} In the study by Scarlett *et al.*,³⁰ pollen exposure did not modify the association of PM₁₀ levels with lung function. In Just *et al.*,¹³ interactions were demonstrated between O₃ and pollen count for asthma attacks. Previous epidemiological studies have suggested an interaction between air pollution and pollen exposure, regarding respiratory and allergic morbidity. Recently, Bédard *et al.*²⁴ showed that associations between uncontrolled rhinitis and pollutants were stronger during grass pollen season, but not during birch pollen season.

Lastly, in our study, significant but slightly lower respiratory parameters were observed in association with grass pollen and air pollution, which may have little or no impact on respiratory health at the individual level, especially in non-asthmatic children. Nevertheless, from a comprehensive perspective, small and clinically unimportant changes in lung function at the individual level may have a substantial impact at the population level, by increasing the proportion of children with clinically low lung function.

CONCLUSION

This study showed a deleterious effect of short-term exposure to both grass pollen and air pollution on the lung function of children from the general population and suggested a synergy between chemical and biological air contaminants.

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Os espaços verdes podem melhorar a saúde pulmonar em zonas de poluição atmosférica baixa a moderada mas não alta: Estudo *Seven Northeastern Cities*

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RESUMO

Introdução Há um interesse crescente no impacto da exposição a espaços verdes nas doenças das vias respiratórias, mas o impacto dos espaços verdes na função pulmonar das crianças é limitado. O nosso objetivo foi investigar as associações entre os espaços verdes envolventes das escolas e a função pulmonar em crianças e se estas associações variam em função da exposição à poluição atmosférica.

Métodos Entre 2012 e 2013, realizou-se um inquérito transversal e uma espirometria a 6740 crianças em idade escolar. Os padrões de função pulmonar foram determinados como volume expiratório forçado em 1 segundo / capacidade vital forçada ((FEV₁/FVC <0.8) ou restritiva (FEV₁/FVC ≥0.8, mas FVC <80% do previsto). Os espaços verdes escolares, conforme definido pelo índice de vegetação da diferença normalizada (NDVI) e índice de vegetação ajustada ao solo. O dióxido de azoto, dióxido de enxofre e concentrações de matéria particulada foram avaliados utilizando um modelo espaciotemporal e dados de monitorização nacionais. Foram utilizados modelos lineares generalizados a dois níveis para investigar associações e interações.

Resultados Em termos gerais, um IQR em NDVI dentro de 500 m foi associado a um FEV₁ mais elevado (+57 ml 95% IC 44 a 70) e FVC (+58 ml 95% IC 43 a 73). O NDVI foi igualmente associado a 25% de redução das probabilidades de restrição espirométrica (OR: 0,75, 95% IC 0,65 a 0,86). Contudo, relativamente às crianças expostas ao quartil mais elevado face ao quartil mais baixo de material particulado, o aumento do NDVI foi paradoxalmente associado ao mais baixo -40 ml de FVC (95% IC -47 a -33, p de interação <0,05).

Discussão Os nossos achados sugerem que, neste estudo, a criação de espaços verdes em zonas urbanas poderá promover a saúde pulmonar em zonas de poluição moderada a baixa, mas não em zonas de alta poluição atmosférica. Se os resultados forem replicados noutros contextos de poluição moderada a elevada, isto evidencia a necessidade de se adotar uma política de espaços verdes flexível.

INTRODUCTION

Given that over half of the world's population now live in urban environments,¹ exposure to air pollution has become an increasingly important public health problem. The WHO estimated that over 80% of people live in areas that exceed WHO air pollution guidelines.² This is especially relevant for children and adolescents are given their developing lungs. On the other hand, 'greening' of cities has become an important part of making urban areas livable, but its impact on health is not yet fully explored, especially in the context of increasing levels of air pollution-related to rapid urbanisation.

Despite the widespread belief that greater greenness is better for general health, the current evidence on associations between greenness and respiratory health is limited.³⁻⁷ A few studies have investigated the link between exposure to greenness and childhood lung function, but the results are not consistent.⁴⁻⁶ Furthermore, there is limited data on the link between school greenness and spirometry measures that are objective indicators of respiratory health and morbidity.^{4,7}

One reason for conflicting results between exposure to greenness and health may be explained by inaccurate capture of exposure to greenness as residential greenness does not capture school exposure where children spend

Key messages

What is the key question?

► Is there any association between greenness surrounding schools and lung function in children, and whether these associations are modified by air pollution exposure?

What is the bottom line?

► Our study of children and adolescents provides evidence suggesting beneficial effects of school greenness on lung function in children in areas with low or moderate levels of particulate matter and sulphur dioxide. These associations reversed in very high-pollution areas.

Why read on?

► Our findings suggest that greening urban areas may promote lung health in low-moderate-pollution areas but not in high air pollution areas. These findings open a new paradigm of having flexible green policies based on the air pollution levels.

most of their day time.⁸ Chinese students are enrolled based on the proximity-based school admission policy, which means that greenness surrounding schools are representative of both school exposure and residential exposure.⁹ This provides an ideal setting to capture the green exposure for children more accurately. Greenness surrounding schools also provide important psychological and social benefits for children. Accessibility to green space surrounding schools, such as active commute, would influence school children's physical activities and time and reduce obesity prevalence,^{10,11} while their influence on lung function was still unclear.¹²

Another issue that needs to be considered in the context of greenness exposure is the interaction between air pollution and greenness. Some studies have reported differential associations between residential greenness and respiratory health for different pollutant concentrations;^{5,13} however, such research has been mostly conducted in low-to-moderately polluted areas such as Europe and North America. More evidence is needed to determine and explore interactions between the effects of greenness and air pollutants across a wider range of pollutant concentrations, including higher concentrations observed in countries like China, such evidence can lead environmental policies.

Given these knowledge gaps, we sought to investigate the associations between greenness and lung function in 6740 school children and to identify whether these associations were modified by air pollution exposure.

METHODS

Study population

We undertook a population-based cross-sectional study between 2012 and 2013, in seven Chinese cities. Study sites



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and participant recruitment details were reported previously.¹⁴ Twenty-four urban districts, including 48 elementary and middle schools, were selected to maximise the range of greenness and air pollutants (online supplemental figure S1 and S2). A four-stage stratified cluster sampling strategy was applied to generate random samples in elementary and middle schools (online supplemental methods 1). Participants who had lived for less than 2 years at their residential address were excluded. Finally, 6740 children who completed the lung function tests and questionnaires were included in this analysis.

All study procedures and protocols were reviewed and approved by the Human Studies Committee of Sun Yat-sen University. Informed consent was given by every children's parent or guardian.

Spirometry

Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were measured using portable electronic spirometers (Spirolab, MIR, Italy). All testing protocols were standardised and undertaken by suitably trained staff in accordance with American Thoracic Society and European Respiratory Society guidelines (online supplemental methods 2).¹⁵

As Global Lung Initiative reference values were not a good fit for our study,¹⁶ we used the predicted values from our previously published Chinese reference equations to define pulmonary dysfunction.¹⁷ Airway obstructive respiratory pattern was defined as $FEV_1/FVC < 0.8$; spirometric restriction by $FEV_1/FVC \geq 0.8$ but $FVC < 80\%$ was predicted. Normal pattern was defined as $FEV_1/FVC \geq 0.8$ and $FVC \geq 80\%$ was predicted.¹⁷

Greenness surrounding schools

Two satellite-based vegetation indices, normalized difference vegetation index (NDVI)¹⁸ and soil-adjusted vegetation index (SAVI),¹⁹ were used to define greenness surrounding each school's boundaries. NDVI and SAVI are measures of greenness that are based on the ratio of visible and near-infrared light reflection. The NDVI and SAVI values range from -1 to +1, corresponding to water (-1), to barren rock (0) and to vegetated areas (+1). SAVI additionally includes a correction factor to suppress soil pixels. Our greenness estimates were derived using two cloud-free Landsat 5 Thematic Mapper satellite images at 30 m resolution (<http://earthexplorer.usgs.gov>) in August 2010. We not only used greenness within a 500 m buffer around a school as our main exposure variable²⁰ but also assessed 300 m and 1000 m buffer sizes in sensitivity analyses. All calculations were performed using ArcGIS V.10.4 (ESRI, Redlands, California, USA).

Air pollution

We focused on particulate matter (PM_1 and $PM_{2.5}$), nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) as the main air pollutants in this study, and our methods have been published previously.²¹ Briefly, air pollution concentrations were measured at the municipal air monitoring station closest to each school's location (<1.5 km). Uniform measurement methods for each pollutant were used, which followed standards from the State Environment Protection Administration of China.²² Daily air pollution concentrations were calculated by averaging hourly observations. In addition to pollutant measurements, we also used satellite-derived exposure estimates for PM_1 , $PM_{2.5}$ and NO_2 . Concentrations were estimated using a spatiotemporal model fitted to link ground-level measurements with aerosol optical depth data and other predictors such as land cover, meteorological variable and calendar month (online supplemental methods 3). The R^2 and root mean squared error for daily $PM_{2.5}$ and PM_1 predictions was 83% and 18.1 $\mu g/m^3$, and 59% and 22.5 $\mu g/m^3$, respectively.²³ The average annual concentrations from 2009 to 2012 were used as surrogates of long-term air pollutant exposures.²⁴ With the exception of SO_2 , we used spatiotemporal model estimates for all air pollution exposure analysis.

Confounders

Potential confounders were selected *a priori* and included: age (year), sex, height (cm), weight (kg), household annual income (≤ 5000 ¥, 5001–10000¥, 10001–30000¥, ≥ 30001 ¥), parental highest educational attainment (no school, primary school, middle school, junior college or higher), environmental tobacco exposure (yes/no), home coal use (yes/no), pet keeping (yes/no), home renovation in 2 years (yes/no), family history of atopy (yes/no), premature birth (yes/no), season of lung function test (spring/summer/autumn/winter). We analysed body mass index (BMI) and physical activity as mediators (Directed Acyclic Graph (DAG) in online supplemental figure S3).

Statistical analysis

To assess the relationship between lung function outcomes and greenness, accounting for district-level effects as random effects, we used two-level generalised linear regression models with fully adjusted variables (online

Table 1 Characteristics of the study participants from the Seven Northeastern Cities study (n=6740)

Characteristic	Value (mean $\pm$ SD, n (%), or median (Q1, Q3))
Age (years)	11.56 $\pm$ 2.07
Height (cm)	154.80 $\pm$ 12.69
Weight (kg)	47.74 $\pm$ 14.93
Sex	
Boys	3382 (50.18%)
Girls	3358 (49.82%)
Parent Education $\geq$ High school	4211 (62.48%)
Family income per year	
$\leq 10\,000$ Yuan	1634 (24.24%)
10 001–30 000 Yuan	2394 (35.52%)
30 000–100 000 Yuan	2437 (36.16%)
$> 100\,000$ Yuan	275 (4.08%)
Season	
Spring	2969 (44.05%)
Summer	2258 (33.50%)
Autumn	838 (12.43%)
Winter	675 (10.01%)
Environment tobacco exposure	3281 (48.68%)
Home coal use	676 (10.03%)
Home renovation	2416 (35.85%)
Pet keeping	1435 (21.29%)
Family history of atopy	1390 (20.62%)
Preterm birth	338 (5.01%)
Exercise time (hours/per week)	7.00 (2.00, 10.00)

supplemental methods 4). Briefly, all models included children as the first level and district as the second level. The linearity of the associations between greenness and lung function was graphically tested through smoothing curves and statistically tested using restricted cubic spline functions. In the main analysis, we hypothesised a linear relationship between greenness measurements and lung function in the main model. Additional adjusted models included variables in the main model as well as individual adjustment for $PM_{2.5}$, PM_1 , NO_2 and SO_2 level, to check whether air pollution confounded the associations with greenness.

To investigate potential effect modification by specific air pollutants (PM_1 , NO_2 and SO_2), we categorised pollution levels into three groups: low (0%–25%), medium (25%–75%) and high (75%–100%) based on quartiles. Stratified and interaction analyses were used to explore the relationships between greenness and lung function in subgroups.

Mediation analysis was used to test the contribution of BMI and physical activity as mediators between school greenness and lung function. The proportion of the mediated effect and its 95% CI were obtained by the mediation package in STATA for causal mediation.^{25,26} In brief, we assessed the mediating effects of BMI and physical activity by calculating the bias-corrected 95% CIs of indirect paths.²⁷ The proportion of the mediated effect was calculated as: (β indirect effect/ β total effect) $\times 100\%$. This is the proportion of increase in lung function explained by higher school greenness that can be attributed to physical activity and BMI.

We performed several sensitivity analyses. First, the main analysis was repeated for both 300 m and 1000 m green buffers around each school. Second, we restricted the analysis to 4032 children who reported walking to school and thus lived nearby.

All statistical analyses were performed using STATA 15.0 (Stata Corp LP, College Station, Texas, USA). A two-tailed p value of less than 0.05 was selected as a criterion of statistical significance.

RESULTS

A total of 6740 participants were included, and the average age was 11.6 years (table 1). Half were girls and 78% were surveyed in spring/summer. Almost half of the participants (49%) were exposed to environmental tobacco smoke and 21% had a family history of atopy. Of the total, n=228 (3.38%) and n=459 (6.81%) participants fulfilled the criteria for an obstructive or restrictive spirometric pattern, respectively (table 2).

Table 2 Characteristics of greenness, air pollution and lung function from the Seven Northeastern Cities study (n=6740)

Characteristic	Value (mean±SD, n (%), or median (Q1, Q3))
Greenness	
NDVI _{300 m}	0.295 (0.214, 0.377)
NDVI _{500 m}	0.312 (0.256, 0.362)
NDVI _{1000 m}	0.283 (0.231, 0.362)
SAVI _{300 m}	0.177 (0.123, 0.214)
SAVI _{500 m}	0.178 (0.141, 0.218)
SAVI _{1000 m}	0.163 (0.130, 0.220)
Air pollution	
PM _{2.5} (µg/m ³)	56.58 (48.86, 59.60)
PM ₁ (µg/m ³)	49.67 (41.74, 54.92)
NO ₂ (µg/m ³)	35.25 (31.75, 45.00)
SO ₂ (µg/m ³)	51.00 (38.50, 57.75)
Lung function	
Obstructive	228 (3.38%)
Restrictive	459 (6.81%)
FEV ₁ (mL)	2464±699
FEV ₁ predicted (mL)	2275±570
FVC (mL)	2626±775
FVC predicted (mL)	2497±592

Obstructive: FEV₁ <0.8; restrictive: FEV₁/FVC ≥0.8 but FVC <80% of predicted. FEV₁, forced expiratory volume 1 s; FVC, forced vital capacity; NDVI, normalised difference vegetation index; SAVI, soil-adjusted vegetation index.

The greenness values estimated by two satellite indices—NDVI and SAVI—were highly positively correlated (correlations ranged from 0.75 to 0.99, online supplemental table S1). Thus, NDVI values were used in our main analysis. In contrast, the correlations between air pollutants and greenness were negative, particularly with PM₁ (online supplemental table S1). Greenness levels were slightly higher in the lowest pollution areas while similar in middle–high pollution categories (online supplemental table S2). No regional clustering effect of greenness, air pollutants and lung function were observed in online supplemental table S3 and figure S4.

Compared with the lower value of NDVI, greater greenness exposure was consistently associated with lower odds for spirometric restriction in both crude and fully adjusted models (table 3). Increasing exposure to greenness was also significantly associated with a higher FEV₁ and FVC (table 3). The observed associations remained similar before and after adjustments for air pollutants, except for PM₁ (table 3). This was related to cancelling out of the opposing observed associations between greenness and spirometric restriction and FEV₁ and FVC outcomes across different levels of PM₁, an interaction that was found to be significant (figures 1 and 2).

We found that air pollutant exposures modified the effects of greenness on lung function outcomes. While greater greenness exposure was associated with higher lung function in areas of low–moderate air pollution, this association was reversed in areas with the highest quartile of PM₁ and SO₂ (figures 1 and 2). Specifically, for those exposed to the lowest quartile of PM₁, an IQR increase in NDVI_{500m} was associated with an increase of 45 mL (95% CI 2 to 89) in FVC. In contrast, for those exposed to the highest quartile of PM₁, an increase in IQR

in NDVI_{500m} was associated with a decrease of 40 mL (95% CI 47 to 33) in FVC (p interaction <0.001). A similar pattern was found for SO₂ exposure. Additionally, the associations between school greenness and spirometric restriction were modified in a similar way. Greater greenness was associated with reduced odds for spirometric restriction among those exposed to the lowest quartile of PM₁ while there was a trend for increased odds among those in the highest quartile of PM₁ exposure (figure 1). In contrast, we did not detect interactions for PM_{2.5} or NO₂ (p interaction=0.214 and 0.073, respectively). Similar results were also found in SAVI (data in online supplemental table S4 and figure S5).

In our mediation analysis, the mediated proportion of the association for physical activity and BMI was less than 2% of the total effect (online supplemental table S5). In the sensitivity analysis, findings for 300 m and 1000 m buffers were consistent with those for the 500 m buffer (online supplemental table S6). Among 4032 children who reported walking to school, the average travelling time was 12 min. Though the relationship between greenness and lung function was similar to main results (online supplemental table S7), the newly observed interactions in this restricted population need to be interpreted cautiously as they were not consistent (online supplemental table S8).

DISCUSSION

To our knowledge, this is the first study to estimate the impact of the interaction between exposure to greenness and air pollution on lung function in a highly polluted area. We observed that higher greenness surrounding schools were associated with higher FEV₁, higher FVC and lower odds for spirometric restriction. These associations were not explained by mediating effects of BMI or physical activity. Interestingly, in this setting, with relatively high levels of pollution, we detected significant interactions between the association of greenness and PM₁ and SO₂ on lung function. While greater greenness remained beneficial in areas with low-to-moderate levels of PM₁ and SO₂, it was associated with lower lung function in areas with high levels of PM₁ and SO₂.

A few previous studies have provided evidence on the impact of greenness on asthma, COPD and spirometry, but the results were inconsistent. Some have reported benefits,^{4–6,28} but others have reported null or even harmful effects of greenness on childhood respiratory health.^{13,29} These conflicting results might relate to different definitions of respiratory outcomes, geographical variation in the constituents of greenness and inconsistent adjustments for potential confounders.

Our novel finding of an interaction between the associations of air pollution levels and greenness is supported by a study of asthma in 22 645 participants in England.³ This study reported that greater urban tree density was associated with reduced rates of asthma hospitalisation, and this protective effect appeared to be stronger in areas with: PM_{2.5} above 13.52 µg/m³, SO₂ above 4.23 µg/m³ and NO₂ above 28.23 µg/m³. It is worth noting that these cut-offs for PM_{2.5} and SO₂ fall within our reference group, the lowest quartile exposure, in which we also found that more greenness was associated with better lung function, whereas for the highest quartile, we found that more greenness was associated with poorer lung function. For most children in our study, exposure to NO₂ was below WHO guidelines, whereas some children were exposed to SO₂ almost two to five times higher than WHO guidelines. Similarly, Sarkar *et al*³⁰ reported that increasing greenness was associated with a higher FEV₁/FVC ratio below the threshold (close to NDVI 75th percentile), while this association was attenuated over in the highest quartile of NDVI. The author explained that highly green areas might increase susceptibility to allergic reactions to pollens. Our findings are also consistent with the study by Tischer *et al*²⁸ who found greater greenness to have a stronger protective effect for a number of respiratory symptoms in rural areas compared with urban areas, likely

Table 3 Two-level generalised linear models for association of NDVI around 500 m buffer of school address (per IQR increase) with respiratory outcomes in 6740 children

Model	Airflow obstruction (n=228)	Spirometric restriction (n=459)	FEV ₁ (mL)	FVC (mL)
Minimal adjusted	0.96 (0.83–1.12)	0.75 (0.65–0.86)	55 (41–68)	61 (45–76)
Full adjusted	0.97 (0.84–1.13)	0.63 (0.53–0.76)	57 (44–70)	58 (43–73)
Full adjusted+PM _{2.5}	0.93 (0.79–1.11)	0.75 (0.61–0.92)	38 (22–54)	35 (17–54)
Full adjusted+PM ₁	0.96 (0.82–1.12)	0.92 (0.72–1.17)	11 (–7–30)	9 (–13–32)
Full adjusted+NO ₂	0.94 (0.79–1.12)	0.75 (0.62–0.93)	34 (18–50)	30 (12–50)
Full adjusted+SO ₂	0.96 (0.83–1.11)	0.64 (0.54–0.77)	56 (43–69)	58 (43–73)

Minimal adjusted model was adjusted for age, sex and height. Full adjusted model added weight, parent education level, family income, environment tobacco exposure, home coal use, pet keeping, home renovation, family history of atopy, prematurity and season.

FEV₁, forced expiratory volume 1 s; FVC, forced vital capacity; NDVI, normalised difference vegetation index.

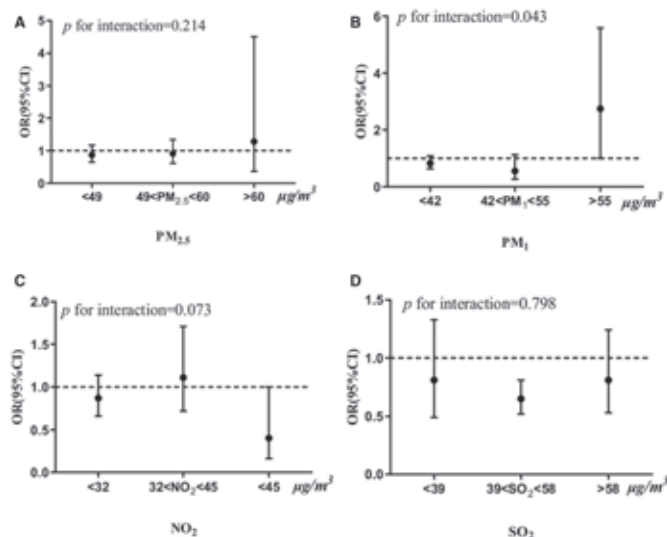


Figure 1 The associations between restrictive lung disease and NDVI in a 500 m buffer around children's school address, stratified by low (0%–25%), medium (25%–75%) and high (75%–100%) ambient PM_{2.5} (A), PM₁ (B), NO₂ (C) and SO₂ (D) concentrations. All models were adjusted for age, sex, height, weight, parent education level, family income, environment tobacco exposure, home coal use, pet keeping, home remodel, family history of atopy, prematurity and season. NDVI, normalised difference vegetation index.

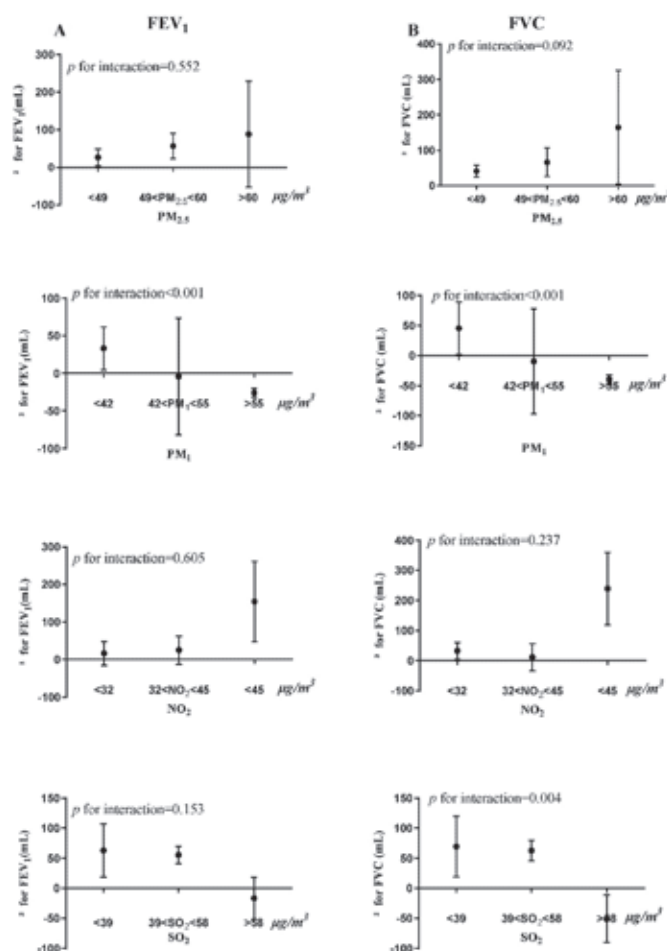


Figure 2 The associations between respiratory parameters and NDVI in a 500 m buffer around children's school address, stratified by low (0%–25%), medium (25%–75%) and high (75%–100%) ambient PM_{2.5}, PM₁, NO₂ and SO₂ concentrations. All models were adjusted for age, sex, height, weight, parent education level, family income, environmental tobacco exposure, home coal use, pet keeping, home remodelling, family history of atopy, prematurity and season. FEV₁, forced expiratory volume 1 s; FVC, forced vital capacity; NDVI, normalised difference vegetation index.

surrogates for low and high ambient air pollution levels, respectively. Together, our findings suggest that greenness may be beneficial when air pollutant levels are moderate, but this association can reverse in the presence of high levels of air pollution. One possible implication of these findings is that planning green spaces in cities should be considered in the context of levels of air pollution and that tree canopy planting policy may be particularly important in countries with high air pollution levels.

We propose several mechanisms that might help explain the positive associations between greenness on lung function for the majority of children schooling in areas with low-to-moderate outdoor air pollution. We found air pollution to be negatively associated with school greenness exposure, consistent with known patterns of increasing urbanisation and decreasing greenness. Our data also provide cross-sectional evidence to support the current hypothesis that greenness can ameliorate the adverse impact of air pollution on children's respiratory health, as we found both consistent and dose-response associations.³ This potential pathway is supported by previous observations that greenness, especially from deciduous trees, can capture particles on their leaves and absorb NO₂.^{31,32}

Green spaces can also encourage physical activity³³ or/and reduce obesity³⁴ although these factors did not explain the link between greenness and better lung function in our study. Increased physical activity related to green space may also be a plausible explanation for the observed adverse impact of greenness on lung function through inhalation of air pollutants in areas with high levels of PM₁ and SO₂; however, our mediation analysis did not support this hypothesis. Another potential mechanism is that air pollutants are likely to induce higher pollen production and increase pollen allergens RNA and protein expression,^{35,36} which in turn can have an impact on lung function. Finally, there is also evidence to suggest that anthropogenic pollutants combined with biogenic volatile organic compounds emitted from trees may produce ozone and PM that are harmful to respiratory health.³⁷ Unfortunately, we did not collect these data and, therefore, were unable to explore these associations.

This study has several strengths. To our knowledge, this is the first study to examine the link between greenness and air pollutions on lung function in school children. The large population representative sample and study setting allowed investigation into effect modification by air pollution levels. Additionally, the results were consistent using two alternative vegetation indices (NDVI and SAVI). Furthermore, we were able to adjust for important confounders although a degree of residual confounding cannot be excluded.

Our study also has some limitations. First, our mediation analysis is limited due to the cross-sectional design, which does not allow for temporality. Second, we used only the school addresses to calculate the pollutant levels and greenness, which might have misclassified the exposure levels for some participants. Limited numbers in some exposure groups also lead to limited power to imprecise estimates of interaction associations. However, Chinese children were reported to spend an average of 8 hours and 11 hours per day in primary and junior high school, respectively.³⁸ It is reasonable to hypothesise that greenspace surrounding schools could be a good proxy for children's exposure. Additionally, our sensitivity analysis including only children who lived near their schools did not change the association trend, and so the impact was likely to have been modest. Third, although we have used two satellite images to provide consistent information on vegetation measurement, these data did not distinguish on vegetation types (eg, trees vs grass and allergenic vs hypoallergenic), structures (parks vs gardens) and quality (lush meadow vs sparse forest) to better classify exposure. However, these errors are likely to be random and shift the associations towards null, and the small numbers in our subgroup analysis may affect the ability to detect positive associations. Another limitation is the lack of separation of the greenspace from bluespace (eg, water), which is represented as negative NDVI values and will underestimate the effect of green space.^{39,40} Given these limitations, our findings should be interpreted as preliminary and longitudinal research is needed to confirm the findings. time that SARS-CoV-2 might be associated with bronchiolitis.

Further studies are needed to explore the association between SARS-CoV-2 and bronchiolitis.

CONCLUSION

Our study of Chinese children and adolescents provides evidence suggesting a positive association between school greenness and lung function in children in areas with low or moderate levels of PM₁ and SO₂. These associations reversed in very high-pollution areas. While the mechanisms behind this paradoxical observation are speculative, if the finding is replicated in other moderate-to-high pollution settings, this highlights a need to have a flexible green space policy, such as a variety of patterns of tree canopies and low pollen producing trees, depending on the background air pollution levels, particularly in settings with high pollution levels.

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Doença do vírus sincicial respiratório grave em bebês prematuros: um caso de imaturidade inata

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RESUMO

O vírus sincicial respiratório (VSR) é o patógeno viral mais comum associado à infecção aguda do trato respiratório inferior em crianças com menos de 5 anos de idade. A doença grave de VSR está associada ao desenvolvimento de complicações respiratórias crônicas, tais como pieira recorrente e asma. Um fator de risco comum para o desenvolvimento de doença grave de VSR é a gestação prematura e isto deve-se, em grande parte, a um sistema imunitário inato imaturo. Isto aumenta a suscetibilidade ao VSR, uma vez que o sistema imunitário inato tem menos capacidade de proteção contra agentes patogênicos numa altura em que a imunidade adaptativa ainda não se desenvolveu completamente. Esta revisão centra-se na comparação de diferentes aspetos da imunidade inata entre bebês prematuros e nascidos a termo para compreender melhor porque é que os bebês prematuros são mais suscetíveis à doença grave do VSR. A identificação de biomarcadores imunitários inatos associados ao desenvolvimento de doença grave do VSR e a compreensão de como estes se comparam entre bebês prematuros e nascidos a termo continua a ser uma questão de importância crítica que ajudaria ao desenvolvimento de intervenções para a redução do fardo da doença nesta população vulnerável.

INTRODUCTION

Respiratory syncytial virus (RSV) is the most common viral pathogen associated with acute lower respiratory tract infection (LRTI) in children.^{1,2} Globally, RSV affects 33.1 million and causes 60 000 in-hospital deaths in children under 5 each year.³ Despite advancements in medical technology and in-hospital care, morbidity and mortality from RSV remains high, particularly in low-income and middle-income countries.³ Initiatives have been put forward by WHO to develop an RSV vaccine and while several RSV vaccines are in late phase trials, the only licensed RSV therapy currently available, palivizumab, is very expensive and only recommended in infants with high-risk comorbidities.⁴

Severe RSV disease has been associated with chronic respiratory complications such as wheezing, decreased lung function and development of asthma even in the absence of indicators of atopic predisposition.^{5,6} Preterm birth (<37 weeks gestation) has been associated with increased susceptibility to severe RSV infection, with preterms over-represented in terms of hospitalisation.¹ While it is difficult to unravel the relative contributions of innate immune system immaturity, variations in cardiopulmonary physiology related to prematurity and structural lung disease (eg, bronchopulmonary dysplasia), innate immune factors are likely to play an important role in determining susceptibility to severe disease.

Primary infection with respiratory pathogens occurs in infancy. As adaptive immunity has not fully matured, the innate immune system plays a crucial role in immune defence in young children and its role in susceptibility to severe disease is a current focus of much research. As preterm infants are at a higher risk of severe RSV disease, development of targeted preventative interventions and therapeutic strategies are urgently needed. This review will focus on how immaturity of the innate immune system in preterm infants predisposes them to more severe RSV disease and aims to guide the development of strategies that may target innate immunity to alleviate disease.

RESPIRATORY SYNCYTIAL VIRUS: VIROLOGY AND IMMUNE RESPONSE

A complex assortment of proteins are required for RSV attachment, the G and fusion (F) proteins and replication, the matrix, nucleoprotein, phosphoprotein and large polymerase protein.⁷ RSV also encodes two non-structural (NS) proteins, NS1 and NS2, which block type 1 interferons (IFN) and prevent the initiation of pro-inflammatory responses to promote viral replication.⁷ The contribution of viral load to disease severity is unclear. While no studies have directly compared viral loads between preterm and term infants, in infants hospitalised with RSV bronchiolitis, increased viral loads have been associated with increased disease severity.⁸ However, in neonatal mice, viral load has been shown to be unrelated to disease severity, instead absence of functional CX3CR1 was the primary factor.⁹

Detection of RSV occurs through host pattern recognition receptors expressed on antigen-presenting cells and epithelial cells.¹⁰ The toll-like receptors (TLRs) 2, 3, 4, 6, 7 and 8 are important during RSV recognition.¹¹ Mouse studies have shown TLR4 can also detect the RSV F-protein, upregulating CCL2 and CCL5 production by macrophages.^{12,13} TLR3 and TLR7/8 detect double-stranded RNA and single-stranded RNA, respectively, and in mice these have been shown to collectively control inflammation within the lungs to reduce mucus production, eosinophilia and hyperplasia of the lung epithelium during RSV infection.^{14,15}

The immune response following RSV infection is crucial to the outcome of disease severity. Primary infection is reliant on early cytokine responses and innate immune cells to control infection and promote appropriate adaptive responses. While the exact causes of pathology during RSV infection are still contested, the data suggest that pulmonary pathology may be mediated through a lack of T-helper cell 1 (Th1) pro-inflammatory responses brought on by innate immaturity.¹⁶ As innate immaturity may be more pronounced in preterm infants, this could further promote immunological phenotypes that allow for severe disease to manifest.

PRETERM INFANTS ARE MORE SUSCEPTIBLE TO SEVERE RSV DISEASE

Preterm infants are born earlier than 37 weeks gestational age (wGA). These infants are further categorised into extreme preterm (<28 wGA), very preterm (28–32 wGA) or moderate-to-late preterm (32–36 wGA). Prematurity, across all gestational ages, is an important risk factor for severe RSV disease. Among preterm infants, intensive care unit admission, length of hospital stay and requirement for mechanical ventilation are highest in extreme preterms, with risk decreasing as gestational age increases.¹⁷ Other risk factors such as chronic lung disease and congenital heart disease are also more prevalent in preterm infants, further contributing to their susceptibility to severe RSV disease.

Chronic lung disease in premature infants is classified as any pulmonary disease that results from a neonatal respiratory disorder.¹⁸ Most commonly, chronic lung disease is the result of bronchopulmonary dysplasia due to immature lung development.¹⁸



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This leads to disruption of alveolar development, gas exchange and reduced capacity for supplemental oxygenation. These factors are exacerbated during RSV infection as airway diameters are further decreased through mucous production, bronchospasm and necrosis of the respiratory epithelium.¹⁹ This can result in long-term wheezing, decreased lung function and asthma when no atopic predisposition is evident.^{5–6} In infants with congenital heart disease and evidence of pulmonary hypertension, mortality and severe disease rates are increased. Progressive inflammation within the lungs resulting in hypoxia and reduced ability to compensate, results in this group being particularly susceptible to severe RSV disease and mortality.²⁰ RSV-related mortality rates from 2003 to 2013 in Canada showed 33.8% were born preterm, 28.2% had congenital heart disease and 18.4% had chronic lung disease.²¹ Additionally, the global RSV-associated mortality in young children (RSV Global Online Mortality Database or GOLD) study, found prematurity and congenital heart disease to be more prevalent in children whose infection resulted in mortality.²²

An immature innate immune system coupled with chronic pulmonary and/or cardiac disease increases susceptibility to severe and life-threatening RSV disease among preterm infants. Thus, strategies to mitigate severe RSV disease should be targeted to this vulnerable population.

WHY IS THE INNATE IMMUNE SYSTEM CRUCIAL IN RSV DISEASE?

Infants rely on maternal antibodies for protection against primary infection due to a lack of adaptive immune responses, however, in RSV disease this is not always effective. Maternal transfer of RSV F-protein specific IgG neutralising antibodies is thought to contribute to preventing infection. These are highest during the first 3 months of life; however, RSV infection is most prominent during this period suggesting an inadequacy of maternal antibody to confer protection alone.^{23–24} Studies have demonstrated that levels of maternal neutralising antibodies to RSV are comparable or lower in preterm infants compared with term infants.^{25–26} In one study, preterm infants born at 29–36 wGA had similar maternal neutralising antibodies to term infants, however, these were lower in those born ≤ 28 wGA.²⁶ This supports the idea that for preterm infants, innate immune responses may also be a critical factor in their ability to control and clear primary RSV infection.

During primary infection, innate immunity ultimately drives the subsequent adaptive response. A Th1-focused pro-inflammatory response is thought to be important for RSV clearance and disease resolution. However, in infants, innate cells have a diminished capacity to present antigen and initiate appropriate pro-inflammatory responses. Historically, these have suggested skewing towards a Th2 response, which is associated with a reduced ability to clear RSV leading to the development of severe disease.^{5–16} This is evident from studies in infants with severe RSV-induced bronchiolitis having a higher interleukin-4 (IL-4)/IFN- γ ratio and reduced Th1-associated cytokine release.^{27–28} However, more recently, susceptibility to severe RSV disease is thought to be associated with reduced Th1 pro-inflammatory responses rather than Th2 skewing, although both aspects are important²⁹ (figure 1).

Notably, genetic predisposition to severe RSV disease primarily involves the innate immune system. Approximately 16% of the susceptibility could be attributed to a group of single nucleotide polymorphisms (SNPs) in STAT2, IFNA5, IFNA13 and IFNAR2.^{30–31} These IFN- α SNPs strongly suggest the importance of innate immunity in the disease pathogenesis given that RSV inhibits type-1 IFN responses through its NS proteins. Additionally, the NS1 protein has been shown to suppress CD8+ and Th17 cells, while promoting the proliferation and activation of Th2 cells.³² Other genetic associations include a SNP in the promoter region of IL-8, important for neutrophil recruitment and polymorphisms in IL-4R α which promotes the differentiation of Th2 cells and may suppress pro-inflammatory responses.^{33–34} It has also been found that two SNPs encoding Asp299Gly and Thr399Ile substitutions in the TLR4 ectodomain are associated with severe RSV disease in preterm infants.³⁵

Together, these data suggest that innate immune system immaturity, especially in preterm infants, is critical in the susceptibility to severe RSV disease.

PULMONARY INNATE IMMUNITY AND RSV DISEASE IN PRETERM INFANTS

The pulmonary environment plays a significant role in controlling RSV infection.³⁶ Many mucosal factors including IgA secretion, the microbiome, surfactant and the production of antimicrobial peptides (AMPs) and cytokines by airway epithelial cells (AECs) contribute to this (figure 2).

Infants rely primarily on nasal breathing during the first year of life. This indicates that mucosal responses in the nasopharynx are the first line of defence against invading respiratory pathogens to prevent transgression to the lower respiratory tract. Studies in preterm and term infants with RSV have shown similar levels of IFN- γ , IL-10 and RANTES (Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted) from nasal swabs, suggesting the upper airway cytokine secretions may not contribute much to the development of severe disease in preterms.³⁷ Mucosal IgA is another key component in protection against RSV. While no studies have compared IgA responses with RSV in preterm and term infants, it has been shown that 56% of preterms (mean 33 wGA) have detectable levels of IgA in saliva at birth compared with 69% of term infants.³⁸ Additionally, during RSV infection, IgA secretion from B-cells is activated through IFN- α , which is decreased in preterm infants suggesting that IgA responses may be diminished, increasing susceptibility to develop severe disease.³⁹

The microbiota is known to play a key role in the innate immune response through the production of AMPs and MyD88 activation.⁴⁰ While microbiota differences between preterm and term infants have not been explored in the context of RSV infection, in children hospitalised with RSV disease there is an enrichment of *Haemophilus influenzae* and *Staphylococcus aureus* in the nasopharynx. This is associated with increased expression of TLR, neutrophil and macrophage activation genes.⁴¹ It has also been shown that prebiotic and probiotic supplementation reduced the risk of viral respiratory tract infections due to rhinovirus, which may be relevant to RSV.⁴² Given the importance of the microbiota in infection, early medical intervention such as antibiotics used to reduce morbidity in preterm infants may play a role in contributing to RSV susceptibility through microbiota dysregulation.

Surfactant proteins A and D (SP-A, SP-D) opsonise viruses to enhance phagocytosis and has been shown to increase RSV clearance in mice.⁴³ In addition, genetic alterations to SP-A has been associated with severe RSV disease.⁴⁴ While no studies have directly compared SP-A and SP-D between preterm and term infants during RSV infection, SP-A and SP-D are decreased in preterms with respiratory distress syndrome compared with term infants.⁴⁵

AMPs produced by AECs have been associated with protection against RSV through inhibiting viral replication and spread.⁴⁶ However, correlations between AMPs and gestational age suggest that concentrations of lactoferrin, calprotectin and cathelicidin are similar between preterm (24–36 wGA) and term infants during respiratory infection.^{47–48} This suggests AMPs alone may not be crucial to reduce RSV disease susceptibility in preterm infants. Production of IFN- λ by AECs is important in regulating mucosal responses to viral infection and has been shown to suppress RSV infection of epithelial cells.⁴⁹ Although no studies have compared IFN- λ between preterm and term infants, it has been shown that increased levels of IFN- λ in infants is associated with severe RSV disease.⁵⁰ Given the importance of IFN- λ in respiratory infection, the contribution of this to severe disease in preterm infants requires further exploration.

INCREASED SUSCEPTIBILITY TO RSV IN PRETERM INFANTS IS DUE TO ENHANCED INNATE IMMATURITY

Innate immunity is a multifaceted defence mechanism requiring appropriate functionality to control infection. Pathogen recognition, antigen presentation, pro-inflammatory cytokine production and cytotoxic responses are equally important in the shaping of adaptive immune responses and the resolution of RSV infection. As such, collective deficits in different components of innate immunity may be associated with the development of severe RSV disease in preterm infants (table 1).

Pathogen recognition

Numerous studies have compared TLR expression and function in cord blood mononuclear cells (CBMC) of preterm and term infants. Cell surface expression of TLR2 and TLR4 and their ability to produce TNF- α and IL-8 have been shown to increase with gestational age, suggesting extreme preterms would exhibit the weakest pro-inflammatory responses to pathogens.⁵¹ However, this is still debated as similar levels of TLR2 and TLR4 expression in preterm (32–34 wGA) compared with term infants has also been reported.⁵² Stimulation with the TLR2 agonist, lipoteichoic acid, and the TLR4 agonist, lipopolysaccharide, resulted in reduced IL-6, IL-8 and TNF- α production in CBMC from preterm infants (24–36 wGA) compared with those from term infants.^{51–53} Given the importance of TLR4 in detecting the RSV-F

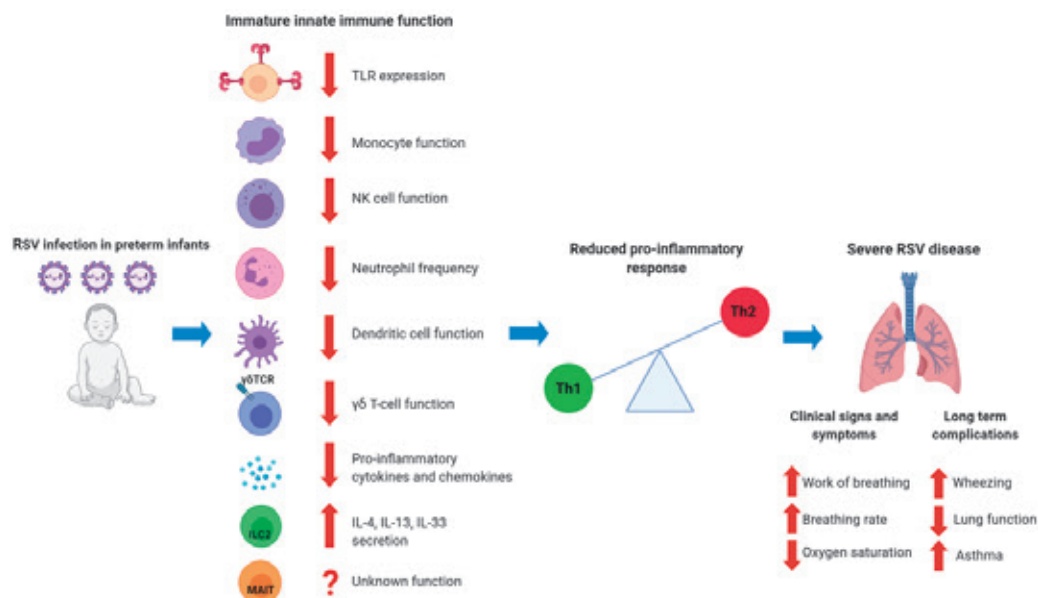


Figure 1 Innate immune response to respiratory syncytial virus (RSV) in preterm infants. Exposure to RSV by preterm infants leads to distinct innate immune responses associated with impaired activation and function of important cell types including monocytes, neutrophils and natural killer (NK) cells as well as potential effects on innate-like unconventional T-cells (eg, mucosal-associated invariant T-cell (MAIT) cells). This leads to reduced T-helper cell 1 (Th1) pro-inflammatory responses contributing to the development of severe RSV disease. This often requires hospitalisation due to the need for respiratory support in the form of supplemental oxygen. Severe RSV disease is also associated with the long-term lung sequelae such as wheezing and asthma. IL, interleukin; TLR, toll-like receptor.

protein, reduced overall TLR4 functionality may limit the ability to initiate early pro-inflammatory responses during infection. Stimulation with resiquimod (R848), a TLR7/8 agonist and nuclear factor kappa B (NF- κ B) activator, led to reduced pro-inflammatory cytokines (IL-1 β , IL-6, IL-12 and TNF- α) in preterm infants (≤ 30 wGA) compared with term infants.⁵⁴ Taken together, these studies suggest a compromised ability to detect viral ligands as well as a reduced antiviral response to control infection through NF- κ B signalling pathway.

Antigen presentation

Monocytes are recruited early during RSV infection to limit viral replication through phagocytosis and are the major producers of IL-6 and TNF- α .⁵⁵ Monocytes have been reported to be either similar or fewer in numbers in preterm infants compared with term infants.^{56,57} Reduced median fluorescence intensity of key antigen presentation markers, HLA-DR, CD80 and CD86 on monocytes was reported in preterm infants⁵⁸ and has been shown to impact the ability of monocytes to present exogenous peptides and activate antiviral CD4+ and CD8+ T-cells.⁵⁹ Monocytes from infants suffering from severe RSV disease have been shown to express less HLA-DR, suggesting a reduced antigen presentation capacity to control viral replication.⁶⁰ Expression of CD14 on monocytes is lower in preterm infants, increasing with gestational age, which is critical because CD14-TLR4 interactions with the F-protein of RSV are essential for innate immune cell activation and chemokine production.^{12,13,53} Such deficits may facilitate the viral escape, further contributing to severe disease susceptibility.

During RSV infection, myeloid dendritic cells (mDCs) can be pathogenic, promoting Th2 responses leading to sustained inflammation and increased epithelial tissue damage.⁶¹ These cells can also be directly infected by RSV leading to increased IL-10 production in addition to enhanced CD80 and PD-L1 expression, thereby reducing T-cell proliferation.⁶² However, the antigen presentation capacity of DCs is largely unaffected by gestational age. Limited data suggest that CD80, CD86 and HLA-DR expression on mDCs is similar between preterms (<33 wGA) and term infants,⁶³ while blood DCs antigen BDCA-1 and BDCA-3 expression on mDCs is also similar.⁶⁴ This is important as BDCA-1/BDCA-3+mDCs express TLR3 and can present viral antigen via MHC class I to promote Th1 responses, activate CD8+ T-cells and natural killer (NK) cells through IL-12 secretion.⁶⁵ Therefore, prematurity appears to effect DCs through their cytokine secretion, influencing subsequent adaptive immunity.

Pro-inflammatory cytokine responses

Production of IFN- α by plasmacytoid dendritic cells (pDCs) is an important mediator of RSV clearance, since host genetic susceptibility to infection and survival of RSV itself all involve attenuated IFN- α levels.⁶⁶ In preterm infants (<32 wGA), secretion of IFN- α in response to viral ligands and CpG-A, a TLR9 agonist, is largely decreased, despite similar frequencies of pDC populations to term infants.^{64,67} This implies a functional deficit in the ability of pDCs to produce IFN- α in preterm infants. In RSV-infected mice, reduced IFN- α responses are associated with reduced pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6 and IL-12) and chemokines (CCL3 and CXCL9 production) while pulmonary viral load is increased and disease severity is augmented.^{68,69} Similarly, in mice, RSV infection of bone marrow-derived DCs upregulated Kdm5b H3K4 demethylase which represses type 1 IFNs and increases production of Th2-related cytokines, IL-4, IL-5 and IL-13.⁷⁰ Bronchoalveolar lavage (BAL) samples from preterm

infants (24–36 wGA) intubated for severe RSV disease have reduced pDC numbers compared with term infants, leading to reduced production of type 1 IFNs and induction of pro-inflammatory Th1 responses.⁷¹ These responses are essential to viral clearance and resolution of infection, therefore, their reduction may allow severe disease to manifest.

Neutrophils are the predominant pulmonary cell type in RSV-induced bronchiolitis, however their role in contributing to disease severity is unclear.⁷² Excessive neutrophil responses are associated with pulmonary pathology and associated with severe RSV disease.^{73,74} The release of elastase, reactive oxygen species and promotion of mucous production leading to pulmonary injury may be more pronounced in preterm infants whose lungs have not fully matured.⁷⁵ The contribution of neutrophil extracellular traps (NETs) to pathology is unclear. NETs are web-like networks of DNA released by neutrophils to trap and eliminate pathogens. They have been shown to contribute to airway obstruction by increasing mucous viscosity during RSV disease⁷⁶ and are often located in areas of alveolar damage,⁷⁷ which may further impact preterm infants suffering from chronic lung disease. A recent RSV human challenge study in adults has shown neutrophil activation prior to infection is associated with symptomatic disease by suppressing mucosal pro-inflammatory responses, especially IL-17A.⁷⁸ This is in contrast to findings from preterms, primarily those born 24–29 wGA, and very low birth weight infants who exhibit fewer neutrophils than term infants.⁷⁹ A potential explanation for this may be in neutrophil activation. Preterm infants (34 weeks median GA) with severe RSV disease had increased neutrophil TLR4 expression compared with term infants, while TLRs 2, 7, 8 and 9 were similar⁸⁰ suggesting heightened ability to detect the RSV-F protein. In bronchoalveolar lavage samples from infants with RSV bronchiolitis, preterm infants exhibit fewer neutrophil numbers than term infants, with numbers comparable to uninfected controls.⁸¹ Nasal washes from preterm infants (33 weeks mean GA) with severe RSV LRTI have lower leucocyte counts and IL-8, a key chemoattractant for neutrophils.⁸² Neutrophils stimulate NK and CD8+ T-cell antiviral responses through antigen presentation and the production of IFN- γ and IL-18.^{83,84} These also promote IL-12 secretion from DCs through CD18/intercellular adhesion molecule 1 interactions to support NK cell maturation and IFN- γ production.⁸⁵ Therefore, preterm neutropenia during RSV infection may lead to weaker activation of key antiviral responses. When combined with the impaired ability of pDCs to produce type 1 IFNs, it may explain why preterm infants are highly susceptible to severe RSV disease.

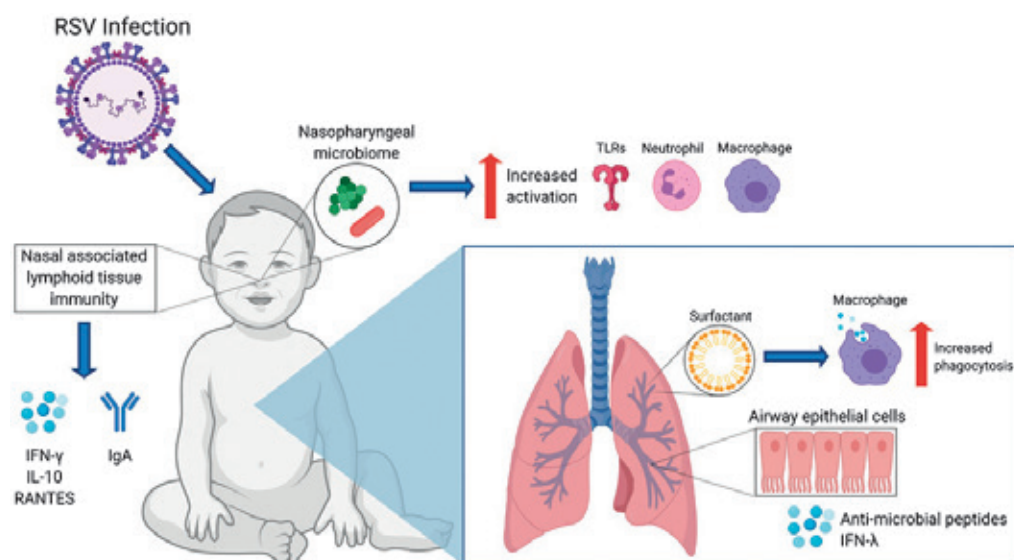


Figure 2 Factors involved in mucosal innate immunity to respiratory syncytial virus (RSV). Nasal-associated lymphoid tissue secrete cytokines and IgA as the first line of defence to invading RSV. The microbiome of the nasopharynx leads to increased toll-like receptor (TLR) expression, neutrophil and macrophage activation. Airway epithelial cells secrete antimicrobial peptides and interferon (IFN)- λ in the lungs to aid clearance of RSV. Secretion of surfactant in the alveoli improves phagocytosis of RSV by macrophages through opsonisation. IL, interleukin.

Cytotoxicity

During early stages of RSV infection, clearance is mediated through NK cells. This occurs through cytotoxic lysis of virus-infected cells through granzyme B and perforin and IFN- γ production directly inhibiting viral replication.⁸⁶ The release of IFN- γ also supports activation and maturation of macrophages, DCs and T-cells. NK cells are less abundant in preterm infants, increasing with gestational age.⁸⁷ CD56^{bright} NK cells which are the major producers of IFN- γ are similar in frequency between adult blood and cord blood.⁸⁸ Additionally, both adults and infants have similar capacity to upregulate IFN- γ production in RSV-infected NK cells, which may suggest this would be similar in preterm infants. The production of cytolytic proteins, perforin and granzyme B are reduced in CD56^{dim} cord blood NK cells compared with adults. These data strongly suggest that the cytotoxic capacity of NK cells may be more important to the outcome of RSV severity as RSV-infected NK cells fail to upregulate perforin.⁸⁶ In addition, expression of NK cell activation markers including killer-cell immunoglobulin-like receptors, NKp46 and DNAM-1 (DNAX accessory molecule-1) were lower in infants while the inhibitory marker NKG2A was higher when compared with adults.⁸⁸ Whether or not these differences are more pronounced in preterm infants is unknown. An increased risk of developing RSV-related LRTIs was associated with increased DNA methylation of the enhancer region of perforin-1 in cord blood suppressing its production.⁸⁹ Given its importance in the cytotoxic capacity of NK cells towards virally infected cells it is likely that immaturity of NK cells contributes to severe RSV disease susceptibility, which may be exacerbated in preterm infants.

CONTRIBUTION OF NOVEL INNATE IMMUNE CELL POPULATIONS TO RSV DISEASE SUSCEPTIBILITY

Research regarding the contribution of innate-like immune cells such as $\gamma\delta$ T-cells, MAIT cells and type 2 innate lymphoid cells (ILC2s) to RSV infection is relatively new. While their properties have not been directly compared between preterm and term infants, these cells may represent unique avenues to explore considering their capacity to present antigen, clear virus and influence the balance of Th1 and Th2 immunity.

$\gamma\delta$ T-cells recognise RSV through TLR3 expression and play a role in limiting viral replication and pulmonary pathology.⁹⁰ During influenza infection, $\gamma\delta$ T-cells have been shown to be protective through their cytolytic and antiviral capabilities.⁹¹ This suggests that these cells may also play a similarly important role in RSV disease. $\gamma\delta$ T-cell functional responses in cord blood are diminished compared with adults, demonstrated by reduced IL-17A and IFN- γ levels following stimulation with RIG-1 agonists, however reduced cytokine levels might be due to their lower frequency in early life.⁹⁰ In mice studies, $\gamma\delta$ T-cells from adult mice but not neonatal mice were able to produce IL-17A and IFN- γ in response to RSV, and this was associated with lower pulmonary pathology

and airway inflammation.⁹⁰ In contrast, following RSV infection in children, there was decreased $\gamma\delta$ T-cell-specific IFN- γ production accompanied by increased Th2 cytokine profiles during the acute phase.⁹² While not significant, IFN- γ secretion in preterm $\gamma\delta$ T-cells was reduced following PMA/ionomycin stimulation compared with term infants,⁹³ which might help to explain the susceptibility of preterm infants to severe RSV infections.

Similar to $\gamma\delta$ T-cells, MAIT cells are also in low abundance in cord blood. These cells are typically activated through the MHC class I like protein, MR1, presenting vitamin B metabolites derived from bacteria and yeast.⁹⁴ However, their role in respiratory viral clearance has not been explored in large detail. The secretion of IL-12 and IL-18 have been shown to activate MAIT cells regardless of MR1 presentation.⁹⁵ These cytokines are produced by monocytes, DCs, neutrophils and macrophages during

RSV infection, which indicates that there may be a role of MAIT cells in the control of RSV infection.⁹⁶ Whether MAIT cells can protect against RSV infection is unknown, although they potentially produce pro-inflammatory and cytolytic proteins including IFN- γ , TNF- α , IL-17A, perforin and granzyme B.⁹⁷ Increased circulation of MAIT cells have been associated with improved recovery following influenza A-induced pneumonia.⁹⁸ Infants with low levels of IL-12 suffer increased severity from RSV infection and monocytes from preterms produce less IL-12 following R848 stimulation, suggesting reduced ability to activate MAIT cells during viral infection.^{28, 54}

ILC2s have also been linked with severe RSV disease in infants and are considered the innate counterpart to Th2 cells. They are primarily located in tissue and rapidly secrete IL-4, IL-13 and IL-33 in the lungs following airway inflammation and promote Th2 responses.⁹⁹ In mice, RSV induces IL-13 secretion from ILC2s, increasing airway mucus production and reactivity.⁹⁹ RSV infection also induced rapid IL-33 secretion, which is critical for ILC2 proliferation in neonatal mice but not in adult mice.¹⁰⁰ A recent study in infants with RSV disease found that IL-4 derived from ILC2s in nasal aspirates was a major risk factor for severe disease in preterm infants.¹⁰¹ Extreme preterm infants expressed more IL-4 and this was associated with reduced IFN- γ production, suggesting that ILC2s inhibit Th1 responses to promote Th2-mediated pathology.¹⁰¹ Overall, it appears preterm infants might be more prone to Th2 skewing due to excessive ILC2 proliferation during RSV infection. Coupled with the increased rate of chronic lung disease in preterm infants, the obstruction of airways through Th2-related pathology may contribute to enhanced susceptibility to severe disease.

DEFINING BIOMARKERS FOR SEVERE RSV DISEASE

Identification of biomarkers that could predict the development of severe RSV disease would be extremely valuable (table 2). While current clinical risk factors are informative, inclusion of additional immunological biomarkers may improve clinical management of high-risk patients. This would be particularly true for those infants with no clinical risk factors who develop severe RSV disease and where the clinical signs at presentation may not accurately predict disease trajectory.¹

In children under 2 years with severe RSV disease, it has been found that IL-6, IL-8 and CXCL4 were elevated in their plasma while CCL5 was decreased.^{102, 103} In nasopharyngeal aspirates (NPAs) from infants with RSV, increased IL-10, IL-33 and ILC2s frequencies and decreased IFN- γ has been identified as a contributor to the development of bronchiolitis.^{101, 104} Increased IL-1 β , IL-4, IL-13 and IL-33 in NPAs were also found in infants with severe disease compared with those with moderate disease.¹⁰¹ In NPAs from children under 5 years of age with severe RSV disease, elevated levels of IL-6, IL-8, TNF- α , IFN- α , CCL2, CCL3 and CCL4 were reported.¹⁰⁵ Additionally in NPAs, increased

Table 1 Comparing innate immune cells in preterm and term infants and the overall effect in RSV disease

Cell type	Role in RSV infection	Differences in preterm compared with term infants	Overall effect in RSV disease	Study
Monocytes	Major producers of IL-6 and TNF- α Detect the fusion protein of RSV to initiate innate immunity Antigen presentation to CD4+ and CD8+ T-cells	Reduced MFI of HLA-DR, CD80, CD86 and less CD14 expression	Reduced antigen presentation capacity Reduced ability to activate antiviral CD4+ and CD8+ responses Reduced ability to detect RSV fusion protein leading to reduced chemokine production	Kurt-Jones, <i>et al</i> ¹² Pérez, <i>et al</i> ⁵⁸ Bertram, <i>et al</i> ⁵⁹ Ahout, <i>et al</i> ⁶⁰
Dendritic cells	MHC class I and class II antigen presentation to promote Th1 responses, activate CD8+ T-cells and NK cells Produce IFN- α to initiate innate immunity and antiviral responses	Similar mDC phenotype pDCs produce less IFN- α and are fewer in numbers	Reduced antiviral capacity and ability to initiate innate immune responses Decreased pro-inflammatory cytokine and chemokine production Promotion of Th2 skewing, leading to increased pulmonary pathology and viral load	Scheffold, <i>et al</i> ⁶³ Schuller, <i>et al</i> ⁶⁴ Kerrin, <i>et al</i> ⁷¹ Cormier, <i>et al</i> ¹¹²
Neutrophil	Phagocytosis and antigen presentation to stimulate NK cells and CD8+ T-cells Produce reactive oxygen species, cytokines and antimicrobial granules	Fewer in numbers and reduced IL-8	Reduced activation of NK and CD8+ T-cells Reduced IL-12 secretion from dendritic cells Contribution to disease severity is unclear	Hosakote, <i>et al</i> ⁷³ Yasui, <i>et al</i> ⁷⁴ Assefa, <i>et al</i> ⁸² Sporri, <i>et al</i> ⁸⁴ Costantini, <i>et al</i> ⁸⁵
Natural killer cell	Cytotoxic lysis of RSV-infected cells Major producers of IFN- γ to inhibit viral replication	Fewer in numbers	Reduced cytotoxic lysis of infected cells and inhibition of viral replication Reduced maturation of macrophages, dendritic cells and T-cells	Pérez, <i>et al</i> ⁸⁷ Luevano, <i>et al</i> ⁸⁸
$\gamma\delta$ T-cell	Produce IFN- γ and IL-17A during early RSV infection	Fewer in numbers and reduced TLR3/7	Reduced IFN- γ production associated with increased Th2 cytokine profiles	Dimova, <i>et al</i> ¹¹³ Gibbons, <i>et al</i> ⁹³ Aoyagi, <i>et al</i> ⁹²
MAIT cell	Unknown role in RSV however, has potential for antiviral activity through cytokine production and cytotoxic lysis	Unknown differences however, preterms produce less IL-12 which is key for MAIT cell activation	Unknown	Marchant, <i>et al</i> ⁵⁴ Pérez, <i>et al</i> ⁵⁸
ILC2s	Secrete IL-4, IL-13 and IL-33 to promote Th2 responses	Increased in frequency and IL-4 production	Inhibition of Th1 and promotion of Th2 responses Major risk factor for development of severe RSV disease	Stier, <i>et al</i> ⁹⁹ Mjosberg, <i>et al</i> ¹¹⁴ Saravia, <i>et al</i> ¹⁰⁰ Vu, <i>et al</i> ¹⁰¹

IFN, interferon; IL, interleukin; ILC, innate-lymphoid cell; MAIT, mucosal-associated invariant T-cell; mDC, myeloid dendritic cell; MFI, mean fluorescence intensity; NK, natural killer; pDC, plasmacytoid dendritic cell; RSV, respiratory syncytial virus; Th, T helper cell; TNF, tumour necrosis factor.

IL-4/IFN- γ and IL-10/IL-12 ratios were found in infants with severe RSV disease.¹⁰⁶ In BAL samples the frequency of pDCs in infants with severe RSV disease was similar to the controls.⁷¹ In peripheral blood, reduced NK cell and CD4 T-cell numbers have also been associated

with the need for mechanical ventilation during RSV infection. This is important as these cells are reduced in preterm infants compared with term infants.¹⁰² Neutrophil abundance in peripheral blood from infants <1 year of age correlates with disease severity during RSV-induced bronchiolitis.⁷⁴ This is related to increased levels of IL-8 found in infants with severe RSV bronchiolitis.¹⁰⁷ However, the contribution of neutrophils to pathogenesis is still debated.¹⁰⁸

Changes in gene expression could be a useful biomarker to predict disease progression, however, the practicality of this is debatable. Genes related to neutrophil activity, inflammation and IFN production are overexpressed in infants with severe RSV disease while genes related to T-cells, B-cells and cytotoxicity were suppressed.¹⁰⁹ Overexpression of prolactin signalling and suppression of cytokine signalling (SOCS) genes, SOCS2, SOCS3 and SOCS5, have been found in infants hospitalised with severe RSV disease. This collectively reduced Th1 and promoted Th2 immunity.¹¹⁰ Downregulation of TNFRSF25 in infants with RSV bronchiolitis also supports the development of Th2 immunity by reducing NF- κ B and IFN- γ production.¹¹¹

Studies to identify biomarkers that can distinguish preterm and term infants with severe RSV disease are urgently needed. While we provide comprehensive data on biomarkers associated with severe RSV disease in infants generally, these may not necessarily reflect biomarkers in infants born prematurely. While no differences were observed between pDCs in infants with or without severe RSV disease, those born prematurely exhibited less pDCs.⁷¹ Similarly, preterms with severe RSV disease exhibited less neutrophils and IL-8 which have both been associated with severe disease in infants generally.^{81, 82} Highlighting potential differences in biomarkers for comparative studies of biomarkers between preterm and term infants will go a long way towards answering key questions about their increased susceptibility to severe RSV disease.

CONCLUSION

Preterm infants are more susceptible to severe RSV disease. Immunological, transcriptomic and genetic data suggest that susceptibility to severe disease manifests as a decrease in pro-inflammatory responses as a result of innate immune system immaturity that reduces viral clearance and mediates pathology. Immunomodulatory interventions

Table 2 Summary of potential biomarkers for severe RSV disease in all infants

Biomarker	Source	Level during severe RSV infection	Study
Cytokines			
IL-1 β	NPA	Increased	Vu, <i>et al</i> ¹⁰¹
IL-4	NPA	Increased	Legg, <i>et al</i> ¹⁰⁶ Vu, <i>et al</i> ¹⁰¹
IL-6	Plasma, NPA	Increased	Brand, <i>et al</i> ¹⁰² Tabarani, <i>et al</i> ¹⁰⁵
IL-8	Plasma, NPA	Increased	Brand, <i>et al</i> ¹⁰² Tabarani, <i>et al</i> ¹⁰⁵
IL-10	NPA	Increased	Garcia-Garcia <i>et al</i> ¹⁰⁴
IL-33	NPA	Increased	Vu, <i>et al</i> ¹⁰¹
IFN- α	NPA	Increased	Tabarani, <i>et al</i> ¹⁰⁵
IFN- γ	NPA	Decreased	Legg, <i>et al</i> ¹⁰⁶ Garcia-Garcia <i>et al</i> ¹⁰⁴
TNF- α	NPA	Increased	Tabarani, <i>et al</i> ¹⁰⁵
Cell type			
ILC2s	NPA	Increased	Vu, <i>et al</i> ¹⁰¹
pDCs	BAL	Unchanged	Kerrin, <i>et al</i> ⁷¹
NK cells	PB	Decreased	Brand <i>et al</i> ¹⁰²

BAL, bronchoalveolar lavage fluid; IFN, interferon; IL, interleukin; ILC, innate-lymphoid cell; NK, natural killer cell; NPA, nasopharyngeal aspirate; PB, peripheral blood; pDC, plasmacytoid dendritic cell; RSV, respiratory syncytial virus; TNF, tumour necrosis factor.

targeting the innate immune system to promote Th1 responses or vaccines designed to elicit key Th1 immune responses are likely to be effective strategies to reduce preterm susceptibility to severe RSV disease. These interventions may be aided by the identification of accurate biomarkers that predict severity, however further research is required to fully elucidate the contributions of innate immune cells and their utility as predictive tools to combat severe RSV disease.

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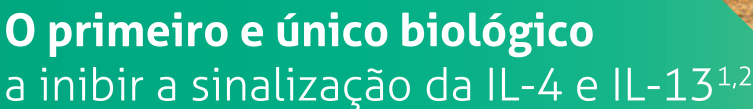
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