

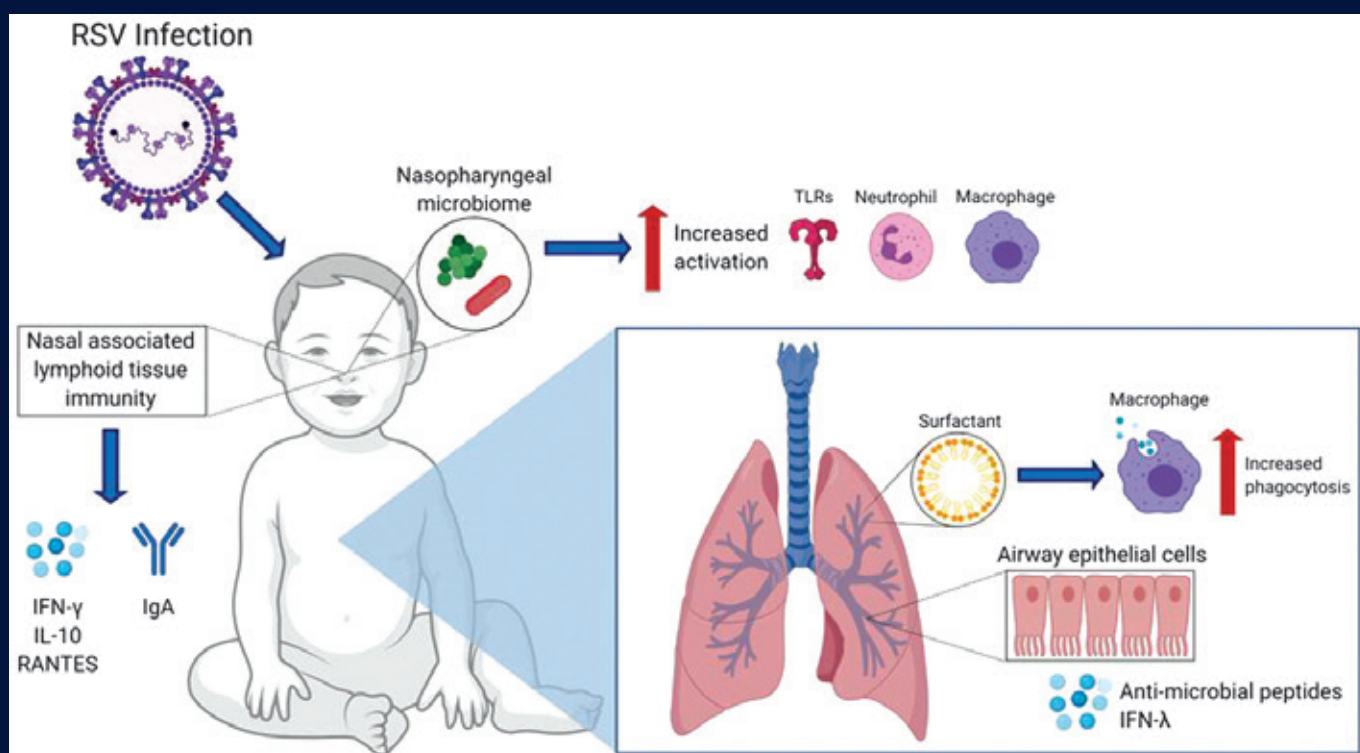
Thorax

ORIGINALITY, RIGOUR & EXCELLENCE IN RESPIRATORY MEDICINE

com artigos do

ARCHIVES OF
DISEASE IN
CHILDHOOD

Edição Portuguesa



1 vez dia¹

Zimbus[®]
breezhaler[®]

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



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

NOVA
ASSOCIAÇÃO
LABA/LAMA
/ICS¹

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 22/04/2021. Disponível em https://www.ema.europa.eu/en/documents/product-information/zimbus-breezhaler-epar-product-information_pt.pdf. Consultado a 21/05/2021. 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: Arandomised, Phase IIIb, non-inferiority study (ARGON). Respir Med. 2020 Aug-Sep;170:106021. 3. Kerstjens HAM, et al. Once-daily, single-inhaler mometasone-indacaterol-glycopyrronium versus mometasone-indacaterol or twice-daily fluticasone-salmeterol in patients with inadequately controlled asthma (IRIDIUM): a randomised, double-blind, controlled phase 3 study. Lancet Respir Med. 2020 Oct;8(10):1000-1012. 4. EPAR: European public assessment report. <https://www.ema.europa.eu> 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), 58 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 tirotóxicose 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 beta₂ (incluindo gotas para os olhos) a menos que existam razões importantes para a sua utilização. **Medicamentos conhecidos por prolongarem o intervalo QTc:** este medicamento deve ser administrado com precaução a doentes que estejam a ser tratados com inibidores da monoamina oxidase, antidepressivos tricíclicos ou medicamentos conhecidos por prolongar o intervalo QT. **Tratamento hipocalcémico:** O tratamento hipocalcémico concomitante com derivados da metilxantina, esteróides ou diuréticos não poupadores de potássio pode potenciar o possível efeito hipocalcémico dos agonistas adrenérgicos beta₂. **Interação com inibidores do CYP3A4 e da glicoproteína P:** A inibição do CYP3A4 e da glicoproteína P (gp-P) não tem impacto na segurança de doses terapêuticas de Zimbus Breezhaler. **Outros antimuscarínicos de ação prolongada e agonistas adrenérgicos beta, 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 cátions orgânicos:** Não é esperada qualquer interação medicamentosa clinicamente relevante. **EFEITOS INDESEJÁVEIS: Frequentes (≥1% a <10%) e potencialmente graves:** hipersensibilidade. **Muito frequentes:** asma (exacerbação), nasofaringite. **Frequentemente (≥1% a <10%):** infeção do trato respiratório superior, candidíase, infeção do trato urinário, cefaleia, taquicardia, dor orofaríngea, tosse, disfonia, gastroenterite, dor musculoesquelética, espasmos musculares, parestia. **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 Medinfa - Produtos Farmacêuticos, S.A. - Rua Henrique de Paiva Couceiro, N° 29, Venda Nova, 2700-451 Amadora Informações Essenciais Compatíveis com o Resumo das Características do Medicamento (ZIM_RCM20210422_IEC_v2).



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Editorial

E lá chegámos novamente à época em que as doenças respiratórias complicam a vida da população e de quem delas cuida. De todos nós... As infeções respiratórias expressam-se com força, em formas agudas, maioritariamente virais. Aumenta o absentismo escolar e laboral. Os recursos aos serviços de urgência aumentam. Internamentos e mortalidade são consequências inevitáveis.

Quem sofre de doenças respiratórias crónicas está em risco. E este risco pode ser mais elevado por circunstâncias de idade ou de comorbilidades. E a pandemia COVID-19, felizmente suavizada pela elevadíssima taxa nacional de vacinação, volta a ser tema de “ordem do dia”. Mais casos, mais internamentos, mais preocupação.

Promoção de saúde, lembrando as regras de etiqueta respiratória que foram tão bem interiorizadas, desinfecção e distanciamento físico sempre que possível, uso de máscaras e vacinação. Contra o vírus influenza, contra estirpes pneumocócicas. E contra o coronavírus. Claro.

Chegou a hora. Não temos muito tempo! O último ano foi suave quanto a “outras infeções respiratórias”, se nos “esquecermos” do flagelo que constituiu a infeção pelo SARS-CoV-2. Mas elas estão de volta. Em força.

A propósito da patologia em idade pediátrica, trazemos neste número para Vossa leitura, duas reflexões. A primeira de Bush e colaboradores sobre a garantia de cuidados a doentes com malformações torácicas congénitas, situações raras que transitam para lá da idade pediátrica, e que por isso mesmo devem ser enquadrados em redes de colaboração que garantam um diagnóstico mais rápido, aumentem a consciência sobre doenças raras, assegurando uma adequada coordenação de cuidados, melhorando o acesso a cuidados e tratamentos especializados. A segunda, de Ian Sinha, a propósito das agudizações de asma em idade pediátrica, sugere que não existem evidências de que a prednisolona administrada na urgência, seja mais eficaz do que a dexametasona, discutindo que existem evidências consistentes de que a dexametasona oral será melhor tolerada.

Em artigos originais inovadores que nos chegam do Reino Unido, Yi Ong e colaboradores discutem os intervalos de referência para o índice de dessaturação de oxigénio de 3% durante o sono em crianças saudáveis com menos de 12 anos, e Chua F e colaboradores refletem sobre fatores de risco de resultados adversos no curso da COVID-19, na estratificação do risco de mortalidade com recurso a pontuações medidas não laboratoriais, no momento da realização dos testes pré-hospitalares do SARS-CoV-2.

Colak Y e colaboradores, num estudo longitudinal de longa duração realizado na Dinamarca, concluem que a insuficiência de vitamina D estará associada, em termos observacionais e genéticos, a um risco acrescido de pneumonias bacterianas, alertando para o efeito benéfico da suplementação desta vitamina, alvo de enorme controvérsia na literatura.

Avaliando o impacto da vacina pneumocócica conjugada 13-valente sobre a ocorrência de empiemas em idade pediátrica na Austrália, Strachan e colaboradores encontraram uma redução significativa dos internamentos por todas as causas de pneumonia bacteriana, associada a um aumento de hospitalizações por empiema, com o aparecimento do pneumococo ST3 como o serotipo dominante, evidenciando a importância dos protocolos de vigilância epidemiológica e a eventual necessidade de adaptação da vacinação.

Também na Dinamarca, por Pape e colaboradores, a partir de um estudo de coorte neonatal, confirmam que uma maior probabilidade dos 3 fenótipos de sibilância/asma infantil já anteriormente descritos, estará associada a experiências adversas ocorridas na infância.

Num artigo de revisão, Ibrahim e colaboradores, levam-nos numa viagem fascinante do que poderá representar a “Breathomics” na nossa prática clínica. Finalmente, Irina Petrache e Esther de Boer, alertam que o “cigarro aquecido” não é mais seguro do que os cigarros de combustão tradicionais, reforçando que a cessação tabágica é sempre o caminho certo.

Boas leituras!

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Eles vão envelhecer: uma rede clínica britânica de doenças raras para malformações torácicas congénitas em adultos

Andy Bush,^{1,2} Rishi Pabary,^{1,2} James Allinson,^{2,3} Matt Hind^{1,2}

A citação de Alexander Pope “*A little learning is a dangerous thing*” (Um pouco de aprendizagem é uma coisa perigosa) (nota: NÃO é conhecimento) é provavelmente uma das frases mais erradamente citadas em toda a literatura, mas aplica-se certamente hoje em dia a muitas doenças raras. Um exemplo é a malformação torácica congénita (MTC, por vezes designada por malformação pulmonar congénita, malformação congénita das vias aéreas pulmonares, malformação adenomatóide quística congénita e uma série de outros nomes exóticos que refletem uma falta de consenso geral no tratamento). Há mais de 30 anos, soubemos pela primeira vez que as grandes MTC podem ser detetadas durante o rastreio das anomalias fetais a meio da gravidez. Sem conhecimento da história natural destas lesões, havia uma moda inicial de intervenções fetais invasivas até se tornar claro que a maioria delas regredia ao ponto de a maioria dos bebés ter nascido bem e não necessitar de intervenção imediata.¹

Aprendemos também que, ainda que a maioria das lesões tenha permanecido assintomática durante muitos anos, podem ocorrer complicações graves em qualquer idade num pequeno número de casos. Estas dividem-se em duas categorias: graves mas tratáveis, tais como infeções, hemorragias e pneumotórax, e as muito raras mas potencialmente letais, nomeadamente a transformação maligna e a embolia gasosa. A falta de conhecimento de quaisquer biomarcadores de resultados adversos, incluindo imagiologia, tem tido o resultado previsível de diferentes centros com diferentes estratégias de tratamento, operando de forma variável em nenhum, todos ou alguns casos. O campo é um deserto de evidências na pediatria e os nossos conhecimentos científicos relativamente limitados sobre a biologia das MTC tornaram o tratamento mais difícil e não mais fácil.

A recente criação de um registo neonatal (<http://www.iclrm.org/>) oferece uma nova oportunidade para abordar esta questão. Uma necessidade é determinar quais as lesões que se tornarão malignas. Algumas contêm anomalias que são pré-malignas ou encontradas em câncros em adultos, incluindo desequilíbrios genómicos, áreas intracísticas e intra-alveolares de proliferação mucinosa, mutações no gene K-ras e instabilidade de microsátélites.²

Outro fator é a DICER1 (*Endoribonuclease Dicer* ou helicase com motivo RNase), uma mutação autossómica dominante na linha germinal localizada a 14q23 com penetração variável. A DICER1 modula o comprimento dos miRNA. A prevalência populacional das mutações é de cerca de 1 em 3700-4600, com 10%-20% de casos de novo. As mutações DICER1 estão associadas ao blastoma pleuropulmonar (BPP) e a um conjunto crescente de outros tumores, incluindo o nefroma cístico, rhabdomyosarcoma embrionário, tumores neurológicos raros como o pineoblastoma, bócio multinodular e tumores gonadais.³ Infelizmente, as características radiológicas não ajudam a distinguir o BPP das lesões benignas, e, mais preocupante, a ressecção não impede o desenvolvimento de outro BPP fora da linha de ressecção. Felizmente, o risco de transformação maligna do BPP com mutações DICER1 é muito menor após os 7 anos de idade.

Outra lacuna no conhecimento é o prognóstico da MTC, tanto recesionada como não. O conhecimento é escasso em pediatria, ou mesmo nenhum, sobre complicações adultas de MTC. Alguns adultos necessitaram de ressecção por presumíveis complicações tardias, mas, sem dados de acompanhamento sistemático, não sabemos o risco de complicações das MTC, a prevalência de intervenção tardia e se, de facto, a ressecção eletiva numa fase inicial da vida seria uma melhor abordagem. Também não sabemos se as causas moleculares subjacentes às MTC também afetam o crescimento do pulmão aparentemente normal circundante e contribuem para o risco de doença pulmonar obstrutiva crónica posterior.

Muitas destas questões também são relevantes para outras lesões congénitas

raras, tais como a hérnia diafragmática. O Quadro de Doenças Raras do Governo do Reino Unido (<https://www.gov.uk/government/publications/uk-rare-diseases-framework/the-uk-rare-diseases-framework>) pode ajudar a abordar estas questões. As quatro principais mensagens são ajudar os doentes a obter um diagnóstico mais rápido, aumentar a consciência das raridades entre os profissionais de saúde, assegurar uma melhor coordenação dos cuidados e melhorar o acesso a cuidados especializados, tratamentos e medicamentos. Em conformidade com isto, foi criada uma rede clínica de doenças raras no CTM pelo NHS England e NHS Improvement. A rede estará sediada no Royal Brompton Hospital e será aberta a qualquer centro que deseje ser incluído. Esta iniciativa é necessária uma vez que, atualmente, existe pouca ou nenhuma especialização focalizada que contribua para o tratamento destes doentes fora dos grandes centros pediátricos.

Adicionalmente, na maioria dos casos, não existe um acompanhamento coordenado ou um contributo especializado após a transição para a vida adulta. Assim, os adultos podem estar a rerepresentar-se com complicações, tais como infeção, ou malignidade em contextos onde a sua história não é avaliada, conduzindo a erros de diagnóstico e de tratamento. A rede visa permitir a todos os doentes com MTC em Inglaterra o acesso a cuidados especializados de qualidade uniforme ao longo da sua vida, reunindo equipas pediátricas e respiratórias de adultos em todo o país.

As MTC são raras e possuem uma histopatologia complexa,⁴ e, como acontece com muitas raridades, os centros individuais podem não ver casos suficientes para acumular conhecimentos substanciais. A rede organizará reuniões de equipa multidisciplinar virtuais para discutir estes casos, tanto para informar o tratamento clínico como para ensinar estagiários. Os casos serão discutidos com especialistas em medicina respiratória, cirurgia torácica, radiologia, patologia e genética pediátricas e de adultos. Atualmente, este serviço existe em Brompton e será oferecido em toda a rede. Esperamos que isto conduza a um consenso nacional sobre o tratamento e, em última análise, ao desenvolvimento de diretrizes nacionais para ajudar as equipas clínicas a tratar doentes com MTC, bem como outras doenças pulmonares congénitas raras.

Na candidatura, a rede propôs três tarefas iniciais. A primeira é a promoção e sensibilização para a rede de MTC. Queremos colaborar com outros centros interessados para estabelecer esta rede. Contactámos informalmente colegas com os quais trabalhamos em estreita

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colaboração em Cambridge, Liverpool e Nottingham, que se mostraram interessados em estar envolvidos. Pretendemos destacar o trabalho da rede com publicações conjuntas de coautoria de pediatria e de adultos em revistas analisadas por pares, apresentações em conferências e através de boletins informativos da *British Paediatric Respiratory Society*, *British Thoracic Society* e *British Lung Foundation*. Esta anotação é o primeiro passo neste processo. Em segundo lugar, queremos formalizar uma via virtual multidisciplinar e transitória de encaminhamento para serviços de adultos destinada a doentes com MTC. Existem muitas vias deste tipo para doenças comuns, por exemplo, fibrose cística, mas não para doenças raras.

Foi proposta uma via até ao ponto de transição,⁵ mas necessita de testes prospetivos, incluindo a contribuição de doentes e famílias. Esta rede nacional terá como objetivo formalizar uma reavaliação detalhada na transição, como anteriormente proposto, das características clínicas, radiológicas, cirúrgicas e patológicas na MTC individual em todo o país. Isto estimulará a normalização dos cuidados com a partilha das melhores práticas. Por fim, queremos estabelecer um registo nacional de MTC em

adultos que será mantido pela rede. O registo fornecerá dados precisos de incidência e prevalência, progresso e resultados clínicos a longo de modo a informar a prestação de cuidados, a alocação de recursos, o planeamento da força de trabalho e a investigação sobre as consequências a longo prazo das MTC.

Para que esta seja uma iniciativa útil, deve haver um planeamento colaborativo e não uma imposição do topo para a base. O principal objetivo deste editorial é procurar a opinião da comunidade pediátrica sobre a melhor forma de desenvolver a rede, sobretudo tendo em conta que não existem recursos financeiros, e sobre como fazê-la funcionar na prática. Um registo deve ter uma finalidade e ser de fácil utilização. Não pode ser um objetivo em si mesmo. Por isso, pedimos aos interessados que nos contactem com as suas ideias. Juntos, podemos começar a preencher as lacunas de evidências no tratamento destas condições raras mas importantes, e converter um pouco de aprendizagem em conhecimento profundo.

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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, 2020. Available at: <https://ginasthma.org/-gina-reports/> (Acedido: março 2021). 4) VestboJ *et al.* Lancet 2016;387:1817-1826. ICS: Corticosteroide inalado; LABA: Agonista β_2 de longa duração de ação.

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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, por inalação, 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 Hiperglicémia **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, dorsalgia, 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** fevereiro 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^a, S.A., -A Av. da Siderurgia Nacional, 4745-457 S.Mamede do Coronado; NIF: 500220913. As Marcas Registradas são propriedade ou licenças das empresas do grupo GSK. (C) 2021 empresas do grupo GSK ou sob licença. DMGMA_PT210310

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As evidências sugerem que a dexametasona é uma melhor opção do que a prednisolona para ataques de asma aguda em crianças

Ian P Sinha^{1,2}

Em crianças com ataques de asma, (1) administrar um esteroide é melhor do que não administrar um esteroide; (2) administrar uma dose de esteroide cedo (de preferência na primeira hora após a chegada) é melhor do que administrá-lo mais tarde; e (3) formar e incentivar as crianças e suas famílias é fundamental. Estes pontos são mais importantes do que o tipo de esteroide oral a utilizar. Apesar disto, espero explicar por que motivo as evidências apoiam a minha opinião de que a dexametasona é uma melhor opção do que a prednisolona para a asma aguda nas crianças. No hospital pediátrico regional em que trabalho, começámos a utilizar dexametasona para a asma aguda em setembro de 2016, como parte de uma atualização das diretrizes locais.

A primeira consideração é a eficácia dos dois medicamentos. A melhor forma de avaliar esta questão é utilizando uma meta-análise de resultados clinicamente relevantes em ensaios aleatorizados controlados (EAC). No desenvolvimento das nossas diretrizes, perguntámos aos pais, enfermeiros e médicos pediatras e de urgência quais os resultados que seriam de maior interesse. Salientaram que a alteração para dexametasona não seria aceitável se fosse menos eficaz do que a prednisolona para a prevenção da admissão em serviço de urgência (SU) ou se implicasse um risco mais elevado de readmissão. Identificámos uma meta-análise de 2014 em que os resultados combinados de seis EAC não revelaram qualquer diferença de eficácia entre dexametasona e prednisolona quando administrada em SU, incluindo a necessidade de admissão durante a visita inicial ao SU ou recidiva de sintomas agudos durante o seguimento de 5 dias ou 2 semanas.¹

Esta revisão sistemática incluiu estudos de avaliação da dexametasona intramuscular, que não é utilizada no Reino Unido, pelo que realizámos a nossa própria meta-análise em 2019.

Identificámos seis EAC (n=1672 crianças de 2-18 anos) comparando a dexametasona oral com a prednisolona em crianças com asma aguda em SU e, mais uma vez, não encontramos qualquer diferença entre os medicamentos em termos de admissão, readmissão não programada ou tempo de internamento.² Embora estes resultados pareçam consistentes, as evidências apresentam falhas. A metodologia e a comunicação dos EAC, em particular no que diz respeito a (1) seleção, medição e comunicação de resultados, (2) regimes de dosagem de esteroides, e (3) medição da gravidade no início do estudo não é uniforme e isto pode excluir a confiança nos resultados agrupados.

Além disso, três estudos foram abertos, existiram evidências de comunicação seletiva de resultados e a precisão é afetada por amplos IC em torno dos resultados de eficácia. A segunda consideração é a segurança e a tolerabilidade, o que é importante, uma vez que a não-inferioridade de um medicamento em relação a outro não é, por si só, uma razão para mudar a prática. A dexametasona é consistentemente demonstrada como sendo mais bem tolerada, com um risco significativamente reduzido de vômitos em SU. Na nossa meta-análise, o risco de vômitos da dexametasona foi de 12 em 850 (1,4%) e o de prednisolona foi três vezes superior (40 de 839 (4,8%)) - isto equivale a um rácio de risco de 0,30 (95% IC 0,16 a 0,55).² Isto é importante porque as sensações desagradáveis como náuseas e vômitos afetam o estado clínico agudo e podem levar a que as crianças atrasem as visitas subsequentes aos hospitais por receio de que lhes seja dado algo que lhes provoque náuseas. A terceira consideração é em torno da duração do curso da prednisolona.

Quando definimos as nossas diretrizes, incorporámos os pontos de vista dos pais. Eles informaram-nos que 3 dias de prednisolona era, muitas vezes, difícil de administrar - quando investigados em estudos, as taxas de adesão aos esteroides orais após a alta do SU variam entre 44% e 85%;³ não existe nenhum estudo de que tenha conhecimento centrado na adesão dos adolescentes à conclusão de um curso de prednisolona prescrito para um ataque de asma, o que é importante

porque eles próprios podem decidir não tomar esteroides orais para minimizar a sua exposição ao medicamento. A quarta consideração, que não tomámos em conta na nossa tomada de decisão, foi o custo (foi o fator menos importante para nós, embora isto seja muito importante para muitas pessoas).

No nosso hospital, o preço de uma dose de dexametasona é cerca de 10%-20% do preço de 3 dias de prednisolona. Compreender o custo destes esteroides é dificultado pela disponibilidade de diferentes preparações e formulações de medicamentos. Felizmente, os especialistas em economia da saúde podem responder-nos a esta questão com algum rigor académico, embora a estimativa de custo-eficácia tenha variado entre estudos com um estudo a favor da prednisolona,⁴ e o outro a favor da dexametasona.⁵

Assim, se as evidências são tão claras, porquê ter este debate? A resposta é simples: as crianças falecem de ataques de asma e é importante que façamos as opções de tratamento corretas. Um argumento contrário à dexametasona é que é difícil de armazenar em cuidados primários. Em retrospectiva, e refletindo sobre o desenvolvimento das nossas diretrizes, o que eu recomendaria a qualquer pessoa que decida fazer esta alteração, é que tente estabelecer uma boa comunicação com os médicos, enfermeiros e farmacêuticos dos cuidados primários, antes e durante a transição.

Esta foi uma oportunidade perdida para codesenvolver uma alteração de prática clínica com todos os intervenientes relevantes, da qual aprendemos - todas as nossas diretrizes respiratórias seguintes têm sido muito mais colaborativas, e são significativamente melhores para esta abordagem metodológica inclusiva. Outro ponto que é frequentemente levantado é que algumas crianças podem, em teoria, requerer cursos mais longos de esteroides orais para tratar a agudização. Esta é uma prática, baseada na ausência de evidências de apoio, que utilizamos muito ocasionalmente no nosso centro em crianças nas quais existem preocupações (baseadas em fatores não baseados em eventos) sobre uma potencial recidiva no espaço de semanas. Poderíamos, por exemplo, dar alta a uma criança com um curso de prednisolona com 2 semanas de diminuição gradual se fossem conhecidas do nosso serviço de asma difícil e se tivessem apresentado uma condição aguda no rescaldo de um controlo muito deficiente da asma durante várias semanas. Esta prática não deve ser generalizada, nem tomada de ânimo leve. No contexto da atual discussão, a utilização destes dois esteroides não os exclui mutuamente: as crianças

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podem ser tratadas com dexametasona de forma aguda e, subsequentemente, receberem alta com um curso de prednisolona.

Existem incertezas sobre os corticosteroides orais para a asma aguda em crianças. É amplamente aceite que a dexametasona é um anti-inflamatório mais potente e com uma meia-vida mais longa do que a prednisolona, mas isto não tem sido avaliado de forma rigorosa nas crianças. À medida que avançamos para uma terapêutica mais personalizada da asma, podemos um dia estar numa situação em que possamos identificar quem responde melhor (e com mais segurança) a um dado medicamento. O meu argumento de que “uma dose de dexametasona é mais fácil do que 3 dias de prednisolona” baseia-se naturalmente na premissa de que uma criança precisa de 3 dias de prednisolona em vez de 1 dia, sendo que estes dois regimes, tanto quanto sei, nunca foram sujeitos a uma comparação direta. O outro fator desconhecido é o risco comparativo

de ambos os regimes de medicamentos no eixo suprarrenal de desenvolvimento de uma criança - será uma dose de um esteroide de ação prolongada altamente potente mais prejudicial do que três doses de um esteroide de média potência? Não sabemos.

Em resumo, não existem evidências de que a prednisolona seja mais eficaz do que a dexametasona, enquanto que existem evidências consistentes de que a dexametasona é melhor tolerada. Num estado agudo em que muito do que fazemos não é de facto motivado por evidências, devemos mudar a nossa prática quando os resultados de EAC e meta-análises apontam todos na mesma direção.

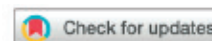
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Estudo observacional para definir intervalos de referência para o índice de dessaturação de oxigénio de 3% durante o sono em crianças saudáveis com menos de 12 anos, utilizando tecnologia de oximetria resistente a movimentos

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RESUMO

Objetivo Definir os intervalos de referência para o índice de dessaturação de oxigénio de 3% (DI3) em crianças saudáveis com menos de 12 anos durante o sono.

Desenho Observacional.

Contexto Casa

Sujeitos Crianças saudáveis entre os 6 meses e 12 anos de idade.

Intervenção Oximetria de pulso noturna em casa. Períodos de sono registados pelos pais. O programa informático *Visi-Download* (Stowood Scientific) analisou os dados com artefactos e removeu os períodos em que as crianças se encontravam despertas.

Principais resultados das medições Foram medidos os seguintes parâmetros de oximetria utilizados na avaliação das condições respiratórias do sono: índices de dessaturação de oxigénio de 3% (DI3) e 4% (DI4) - o número de vezes por hora em que a saturação de oxigénio cai pelo menos 3% ou 4% em relação aos valores iniciais, saturações médias (SAT50), saturações mínimas (SATmin), índice delta 12 s (DI12s) e tempo percentual com saturações inferiores a 92% e 90%.

Resultados Setenta e nove crianças foram submetidas a oximetria de pulso para uso domiciliário noturno, dos quais 66 estudos foram considerados para análise. Os valores medianos para DI3 e DI4 foram de 2,58 (95% IC 1,96 a 3,10) e 0,92 (95% IC 0,73 a 1,15), respetivamente. Os percentis de 95º e 97,5º para DI3 foram 6,43 e 7,06, respetivamente, o que informa o nosso valor de cut off para a normalidade. Os valores médios para o SAT50 e SATmin foram 97,57% (95% IC 97,38% a 97,76%) e 91,09% (95% IC 90,32% a 91,86%), respetivamente.

Conclusão Em crianças entre os 6 meses e os 12 anos de idade, definimos a normalidade do índice de dessaturação de oxigénio de 3% como <7, utilizando oxímetros de pulso autónomos e resistentes ao movimento com tempos médios curtos.

INTRODUCTION

Pulse oximetry is a non-invasive method of estimating blood oxygen saturations and is frequently used in children to investigate sleep-disordered breathing (SDB). SDB conditions may be broadly split into two groups—obstructive sleep apnoea (OSA) and central sleep apnoea (CSA). Urschitz *et al* previously published normative oximetry data in primary school age children.¹ They published mean oxygen saturations (SAT50) and the 4% desaturation index (DI4)—the number of times per hour where the oxygen saturation falls by at least 4% from baseline. These oximetry parameters are frequently used to detect abnormality. Based on their data, a DI4 of >4 is regarded as abnormal and has been widely used as a criterion of abnormality.

In 2012, the American Academy of Sleep Medicine updated the scoring criteria for SDB such that apnoeas and hypopnoeas were defined according to oxygen desaturation of 3% (DI3) from

What is already known on this topic?

- ▶ A 4% oxygen desaturation index greater than 4 is widely used as a criterion of abnormality.
- ▶ Changes in the definition of sleep apnoea (American Academy Sleep Medicine) has resulted in a shift to report 3% rather than 4% oxygen desaturations.
- ▶ Data are lacking on reference ranges for 3% oxygen desaturation indices using modern oximeters able to exclude motion artefact and which use short averaging times.

What this study adds?

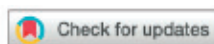
- ▶ This study defines 3% oxygen desaturation index reference ranges for children aged 6 months to 12 years using motion-resistant oximeters with short averaging times.
- ▶ This study supports existing data on commonly reported oximetry variables used in clinical practice for children.
- ▶ Younger children demonstrate higher 3% oxygen desaturation indices, but further study is warranted to establish normative ranges in children under 2 years of age.

baseline rather than 4%.² Alongside this, the 2019 Australasian Sleep Association guidelines on pulse oximetry recommended reporting 3% rather than 4% desaturations when defining cut-offs for normality.³

We previously reported normative oximetry parameters including the DI3 for healthy infants under 4 months of age using modern oximeters. These are able to extract motion artefact and have short averaging times and can therefore accurately detect brief periods of desaturation often seen in children with SDB.⁴ Currently, no data exist on reference ranges for DI3 for older children using these oximeters. This study aimed to provide reference ranges for DI3 in healthy children between the ages of 6 months and 12 years using Masimo oximetry set at a short averaging time.

METHODS

Healthy children of health professionals at University Hospital Southampton NHS Foundation Trust below the age of 12 years were recruited for study. Basic demographics, medical history data for the child and written informed consent to part-



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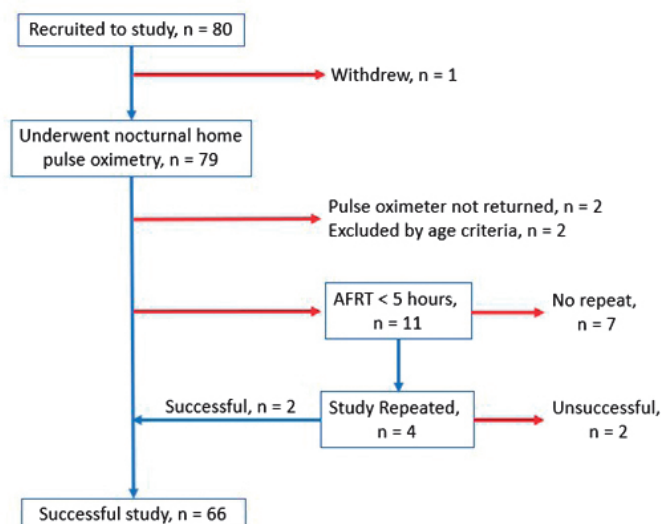


Figure 1 Flowchart showing recruitment. AFRT, artefact-free recording time.

Participants in the study were obtained from the parents. Parents were also asked to complete a sleep history questionnaire for their child. Participants were excluded from the study if they had known respiratory or cardiac conditions or conditions predisposing to SDB. Recordings were undertaken when the child was clinically well and without intercurrent illness.

Data were collected overnight at home using a Masimo Rad-8 (USA) pulse oximeter with a 2s averaging time. Parents were asked to record times of sleep onset, morning waking, periods of wake throughout the night and how the sleep quality compared with a usual night's sleep. Data were collected for the following parameters: mean oxygen saturations (SAT50), minimum oxygen saturations (SATmin), number of oxygen desaturation events >3% (DI3) and >4% (DI4) from baseline per hour, delta index (DI12s), and percentage of time with saturations below 92% and 90%. McGill Oximetry Scores were calculated for participants over the age of 12 months as a screening tool for OSA.⁵

Data analysis was performed using Visi-Download software (Stowood Scientific, UK). The software automatically extracted artefacts caused by low signal quality and movement. Periods of awakening were removed according to data provided by the caregiver and review of the study by a physiologist skilled at interpreting oximetry traces. Previous inter-rater analysis in our laboratory has demonstrated excellent inter-rater reliability for determining artefact-free recording time (AFRT).⁴ Analysis was performed on studies with at least 5 hours of AFRT,¹ which must have included at least two periods of presumed active or rapid eye movement sleep as determined by increased heart rate variability. Each sleep report was reviewed by a paediatric respiratory consultant to ensure there were no abnormalities suggestive of clinical disease that warranted follow-up.

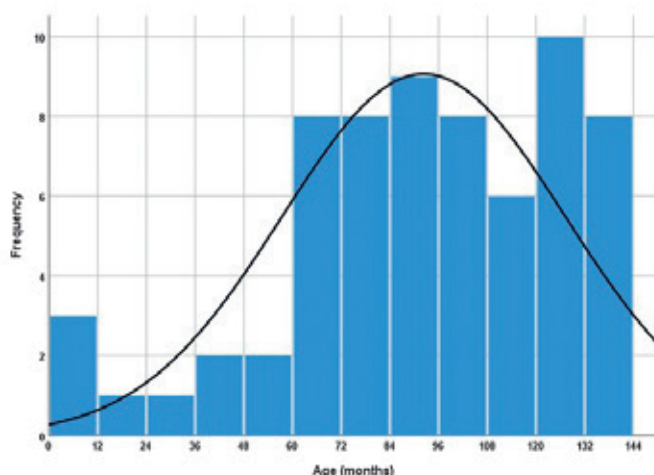


Figure 1 Histogram showing the age distribution of the study participants.

Data analyses were performed using SPSS (V.24) and CI analysis software packages. Normally distributed variables were reported as means and 95% CIs and non-normally distributed variables as medians and 95% CIs.

RESULTS

Seventy-nine children who were recruited to the study underwent nocturnal home pulse oximetry (see figure 1). Two participants were excluded as they did not return the pulse oximeter before the end of the data collection period. During the study collection period, an increase in the evidence base demonstrated that young infants have rapid changes in their desaturation indices with maturation in the first 4 months of life.⁴ Since only two infants were recruited under 4 months of age, a decision was made to exclude them from further analysis. Overall, nine participants were excluded for failing to collect at least 5 hours of AFRT, despite attempts to repeat studies (figure 1).

The median age at recording of the 66 successful participants was 94 months (95%CI 83 to 107 months). The age distribution of participants is displayed in figure 2.

There were 31 male (47%) and 35 female (53%) participants. Body mass index was recorded in 60 participants with a mean z score of -0.03 (95% CI -0.29 to 0.24). The mean duration of AFRT was 532min (95%CI 511 to 553 min). Answers from the sleep history questionnaire revealed 17% of participants were known to snore, which is within the reported normal prevalence in children, and only 3% of participants reported symptoms of SDB. All children over the age of 12 months had a McGill score of 1 which is considered negative or inconclusive for OSA.^{3,5}

For the entire study group, the mean values for SAT50 and SATmin were 97.57% (95% CI 97.38% to 97.76%) and 91.09% (95% CI 90.32% to 91.86%), respectively (table 1). The median values for DI3 and DI4 were 2.58 (95% CI 1.96 to 3.10) and 0.92 (95% CI 0.73 to 1.15), respectively. The 95th and 97.5th centiles for DI3 were 6.43 and 7.06, respectively, which inform our cut-off value for normality (table 2). The mean DI12s was 0.35 (95% CI 0.33 to 0.38).

A subgroup analysis of participants aged between 6 months and 24 months ($n=4$) demonstrated higher DI3 (4.1, range 2.93 to 6.61) compared with the older age subgroup of 24 months to 12 years (2.51, 95%CI 1.93 to 2.93). Using the Mann-Whitney U test, the difference in DI3 between the age subgroups was found to be statistically significant ($p=0.04$). Comparison of DI4 between the subgroups was also found to be significantly higher in the younger age subgroup ($p=0.04$).

DISCUSSION

This study shows that, compared with day workers: (a) people working permanent nights had higher adjusted odds of moderate-severe asthma; (b) people doing any type of shift work had higher adjusted odds of wheeze or whistling in theK, with detailed medical history, current employment information, lifestyle information and demographic details, all of which were collected in a uniform manner. Of these >160 000 also had genetic data available. In addition, >100 000 individuals from the original cohort also provided a detailed employment history.

The study also has some limitations. First, UK Biobank participation rates were low at ~5%, which may have introduced selection bias towards more healthy individuals.²⁹ In fact, the overall prevalence of asthma in all participants studied here was ~5% (also ~5% in the shift worker cohort alone) compared with ~10% within the general population of the UK.³⁰ The UK Biobank provides no data on younger people and only limited data on ethnic minorities. The sample sizes were small for the morning and evening chronotype analyses, which resulted in low power. There was a reduction in sleep duration reported by night shift workers; this would be a potential confounder and so we took self-reported sleep duration into account in Model 3. In fact, we found that Model 3 did not significantly alter the results from Model 2. Furthermore, we determined differences in job type between day workers and shift workers and it is possible that these differences could account for some of our findings. To mitigate this risk, we took into account participants in jobs that might lead to occupational asthma or that required a medical assessment (selecting against asthma).

Table 1 Descriptive statistics for oxygen saturation baseline parameters for the entire study group (n=66): minimum oxygen saturations (SATmin) and mean oxygen saturations (SAT50)

Parameter	Mean	SD	Mean 95% CI	Median	2.5th centile	5th centile
SATmin (%)	91.09	3.20	90.32 to 91.86	92.00	81.40	84.35
SAT50 (%)	97.57	0.79	97.38 to 97.76	97.51	95.81	96.17

CONCLUSION

Motion-resistant oximeters with short averaging times are increasingly recommended in the investigation of children with SDB since they are highly sensitive for detecting brief changes in oxygen saturation frequently seen in these patients.^{6,7} In 2012, the American Academy of Sleep Medicine guidelines were amended such that scoring criteria for OSA and CSA were based on a desaturation of 3% from baseline rather than 4%.² Thus, the number of 3% desaturation events per hour may now be considered more relevant than 4%. While data exist for many parameters of oxygen saturation, little is known about normal limits for 3% desaturations in children using standalone oximeters with short averaging times able to exclude motion artefact. This is the first study reporting reference ranges for 3% oxygen desaturation indices for children older than 6 months using this technology.

Saito *et al*⁸ investigated the role of standalone pulse oximetry in determining indications for adenotonsillectomy in children with SDB. The study included 25 healthy children as controls against 225 participants of interest. In the control group, they reported a mean SATmin value of 94.56% (SD $\pm$ 2.38) while in our population it was 91.09% (SD $\pm$ 3.20). Their reported mean values for DI4 and DI3 were 0.21 (SD $\pm$ 0.29) and 0.74 (SD $\pm$ 0.65), respectively, which are both significantly lower than our data. These discrepancies from our data are most likely explained by the difference in pulse oximeter averaging times used. Saito *et al* used oximeters with a longer averaging time of 5s where pickup of desaturations is often blunted by the increased interval between averaging points.^{8,9} The impact of different technologies has the potential to affect management plans. For example, in this study, the authors concluded that children with a DI3 of more than 3.5 should undergo adenotonsillectomy given the sensitivity for a successful surgery was 94% at this cut-off value. However, this proposed DI3 cut-off value for surgery in SDB would fall well within our reported normative range (mean 2.90 SD $\pm$ 1.65).

Other studies have reported DI3 incorporated into polysomnography (PSG). The largest of these published by Scholle *et al*¹⁰ recruited 209 healthy children for the generation of normative cardiorespiratory parameters using PSG. The study merged results from the Nonin oximeter incorporated into the Embla system and Masimo data obtained from the Alice system. However, unpublished data from our laboratory have previously shown poor limits of agreement between integrated Nonin and standalone Masimo oximeters. Details on averaging times for the oximeters were not reported in this paper. The participants were stratified into eight groups each containing fewer than 35 children. The study is therefore limited by small sample sizes in each group and an absence of reference ranges for the group as a whole. Similarly, Wang *et al*¹¹ again merged data from two separate oximeters and do not report averaging times. Finally, a study by Verhulst *et al*¹² used a Palco pulse oximeter that has not been validated for continuous monitoring in children. These studies highlight how differences in oximeter technology and study protocol are a key limitation in comparing normative values for desaturation indices derived from oximetry or polysomnographic studies within the literature currently.¹³

The decision to recruit a community-based study population was based on the fact that most clinical pulse oximetry traces are undertaken at home. Therefore, the aim was to produce normative data which would be relevant to standard clinical practice. The limitations of this technique are that interpretation of the sleep-wake state is cons-

trained and dependent on parental reporting. Within our laboratory, the physiologists are skilled at interpreting these studies and previous inter-rater analysis has demonstrated excellent inter-rater reliability.⁴

Similar to the Wang *et al*¹¹ and Verhulst *et al*¹² papers, this study recruited children of hospital employees. This may have resulted in recruitment of families who were highly invested in research outcomes. However, the study recruited families from a wide range of healthcare backgrounds creating a diverse group of participants. In addition, our reported standard oximetry parameters are comparable with those generated from polysomnographic studies.¹⁰

Young infants demonstrate a higher frequency of desaturation events.⁴ A subgroup analysis of participants aged between 6 months and 24 months demonstrated higher DI3 compared with the older age subgroup of 24 months to 12 years, an observation explained by the underlying immaturity of respiratory control mechanisms during sleep in normal healthy infants.¹⁴ In our study, the difference in DI3 between the age subgroups was found to be statistically significant ($p=0.04$), despite the fact that we were only able to recruit four infants into the younger age group (figure 2). Further study is warranted to establish normative desaturation indices stratified by age using oximeters with motion-resistant technology and short averaging times throughout infancy.

In conclusion, this study reports for the first time 3% oxygen desaturation indices in children aged 6 months to 12 years using a standalone pulse oximeter with short averaging times able to exclude motion artefact. Based on our data, we define normality as DI3 <7. Our study protocol is in line with the latest technical recommendations for the investigation of SDB in children. We believe that these results can form the basis of reference ranges on which a judgement of normality or otherwise can be determined.

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Table 2 Descriptive statistics for oximetry desaturation events and indices for the entire study group (n=66): number of oxygen desaturations >4% and >3% from baseline per hour (DI4 and DI3), delta index 12 s (DI12s) and percentage time with saturations below 92% and 90%

Parameter	Mean	SD	Mean 95% CI	Median	Mean 95% CI	90th centile	95th centile	97.5th centile
DI4	1.14	0.84	0.93 to 1.34	0.92	0.73 to 1.15	2.60	3.00	3.38
DI3	2.90	1.65	2.50 to 3.29	2.58	1.96 to 3.10	5.65	6.43	7.06
DI12s	0.35	0.09	0.33 to 0.38	0.34	0.31 to 0.38	0.48	0.53	0.57
Time <92%	0.02	0.03	0.01 to 0.03	0.00	0.00 to 0.01	0.05	0.11	0.15
Time <90%	0.005	0.015	0.002 to 0.009	0.00	0.00 to 0.00	0.01	0.05	0.07

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ARTIGO ORIGINAL

Prognóstico precoce da COVID-19 como orientação da hospitalização versus monitorização em ambulatório usando uma pontuação de previsão de risco no ponto de teste

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RESUMO

Introdução Os factores de risco de resultados adversos na COVID-19 estão definidos, mas ainda não existe uma estratificação da mortalidade com recurso a pontuações medidas não laboratoriais, particularmente no momento dos testes pré-hospitalares do SARS-CoV-2.

Métodos Foi utilizada uma regressão multivariada com bootstrap para identificar os preditores independentes de mortalidade em pacientes agudos admitidos em hospital com um diagnóstico confirmado de COVID-19. As previsões foram validadas externamente numa grande amostra aleatória da coorte ISARIC (N=14 231) e numa coorte mais pequena da Aintree (N=290).

Resultados Foram recrutados 983 pacientes (idade mediana 70 anos, IQR 53-83; mortalidade hospitalar 29,9%) durante um período de estudo de 11 semanas. Através de modelação sequencial, foi desenvolvida uma pontuação de cinco preditores denominada SOARS (SpO₂, Obesidade, Idade, Taxa respiratória, História de AVC) para correlacionar a gravidade da COVID-19 através de estratos baixos, moderados e altos de risco de mortalidade. A pontuação discriminou bem a morte hospitalar, com uma área sob os valores característicos de funcionamento do receptor de 0,82, 0,80 e 0,74 na derivação, Aintree e coortes de validação ISARIC, respectivamente. A sua precisão preditiva (calibração) em ambas as coortes externas foi consistentemente mais elevada em pacientes com doenças mais ligeiras (SOARS 0-1), sendo os mesmos indivíduos que podiam ser identificados para monitorização ambulatória segura. A previsão de um resultado não fatal neste grupo foi acompanhada por uma sensibilidade de pontuação elevada (99,2%) e um valor preditivo negativo (95,9%).

Conclusão A pontuação SOARS utiliza características individuais constitutivas e facilmente avaliadas para prever o risco de morte por COVID-19. A aplicação da pontuação poderia potencialmente informar a triagem clínica em cenários de pré-admissão em que a tomada de decisão expedita e fiável é fundamental. O ressurgimento da transmissão do SARS-CoV-2 proporciona uma oportunidade para validar e actualizar ainda mais o seu desempenho.

INTRODUCTION

Rapid and accurate prediction of the probability of adverse clinical outcomes is central to the management of global outbreaks of infection.¹⁻³ Stratification by predicted risk, most commonly for death, can support clinical judgement and potentially assist clinicians in community settings to decide how urgently to refer patients to hospital. Used appropriately, predictive scores can also help inform treatment-related decision-making. The pandemic caused by SARS-CoV-2 lends itself to predictive modelling by having a large at-risk population and a high adverse event rate including death.⁴

Although the recent incidence of COVID-19 has decreased in some parts of the world at the time of

Key messages

What is the key question?

► Can patients with COVID-19 be risk stratified in the prehospital setting without laboratory-measured data?

What is the bottom line?

► A five-predictor risk prediction score (SOARS) based on demographic and clinical characteristics can quickly and reliably identify COVID-19-positive patients who have a low probability of mortality for outpatient monitoring and management.

Why read on?

► Information from the prognostication of SARS-CoV-2-infected individuals early in their illness can be used to guide clinical decision-making with respect to the level of subsequent care.

writing, many countries are already experiencing a 'second wave' of new cases.⁵⁻⁸ An increase in incident cases in many localities is already evident in the UK. It is widely anticipated that viral transmission will continue to surge in the months ahead, particularly with the onset of winter in the northern hemisphere. Not all patients infected with SARS-CoV-2 will require hospitalisation but even among those who initially experience mild symptoms, a sizeable proportion remain at risk of subsequent life-threatening clinical decline. The availability of a practical prehospital predictive tool to triage patients for safe discharge to an outpatient (virtual) monitoring system versus direct admission to hospital for observation or treatment would be highly advantageous.

Reliable prediction tools to differentiate between levels and sites of clinical care already exist and have been successfully implemented in prehospital practice. For example, both the CURB-65 and the CRB-65 scoring systems for the assessment of community-acquired pneumonia include recommendations for out-of-hospital care.^{9 10} Recent research by the ISARIC-4C Consortium has provided an accurate tool to similarly prognosticate for COVID-19-attributed death in hospitalised patients but its reliance on laboratory-measured indices limits its applicability outside the institutional environment.¹¹ Prognostic evaluation of individuals with suspected SARS-CoV-2 infection at the time of diagnostic testing is potentially achievable but has yet to be examined.

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Our objective was to develop and evaluate an easy-to-apply and accurate prognostic score to predict mortality and aid early clinical decision-making by identifying patients infected with SARS-CoV-2 who might benefit from an urgent hospital assessment. To develop the initial risk score, we used multivariate logistic regression to explore the relationships between a large panel of candidate predictors and COVID-19 death. Iterative modelling resulted in a pragmatic predictive score based on five widely available patient variables. The scoring of patients against these selected predictors permitted three distinct risk classes to be defined. The performance of the score was then assessed against two validation cohorts—a large subgroup of the ISARIC study patients and a smaller single-hospital cohort, the latter to better reflect local population characteristics and practice.

METHODS

Study design and characteristics of the derivation cohort

All individuals aged 18 or older who tested positive for SARS-CoV-2 nucleic acid by real-time reverse transcriptase PCR between 1 March and 16 May 2020 after presenting to the emergency department (ED) at Watford Hospital, West Hertfordshire NHS Hospitals Trust were prospectively recruited. Baseline clinical characteristics and investigation results were collected according to a prespecified protocol. Patients were either referred to the virtual hospital (VH) for outpatient monitoring or admitted to a medical ward.

Laboratory, physiologic and radiographic data

All laboratory tests were performed as part of routine clinical care. Nasopharyngeal mucosal swabs for rRT-PCR were couriered to the regional UK Public Health England laboratory. Baseline vital observations included all the parameters recommended by the National Early Warning Score.¹² Chest radiographs acquired in ED were collated and scored at the end of the recruitment period.

Location and level of care

After presentation, patients who were clinically judged to have mild illness were referred to the VH for subsequent monitoring. To avoid missing early clinical deterioration in the postassessment period, they were observed for up to 24 hours in hospital. Patients who remained admitted after the first 24 hours but who did not require additional respiratory support beyond wall-based oxygen were managed on designated medical wards. Where clinically indicated, continuous positive airway pressure (CPAP) was provided on such wards or on the intensive care unit (ICU); intubation and mechanical ventilation were undertaken on the ICU.

Identifying predictors of death in the derivation cohort

The primary outcome of the study was in-hospital death. TRIPOD (Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis) recommendations were followed for multivariate model evaluation and reporting.¹³ Seventy-five baseline clinical and non-clinical variables were initially collected based on their reported association with COVID-19 and analysed by univariate and multivariate logistic regression with bootstrap resampling.^{14,15} Of these, variables with numerically small ORs or a *p* value of >0.05 were not included in the final analysis. Candidate predictors of death were assessed for potential clustering effects and missing at random values were addressed by multiple imputation with chained equations (MICE),¹⁶ with 10–20 random draws to account for data variability.

Development and external validation of the clinical risk score

The large external cohort comprised a randomly selected subpopulation of the ISARIC 4C derivation population (N=20000 provided; 14231 with complete data for scoring). The primary data of these individuals were submitted by 260 hospitals across England, Scotland and Wales to the prospective ISARIC WHO Clinical Characterization Protocol UK (CCP-UK) study.¹¹ We also tested our score against a smaller population of SARS-CoV-2-positive cases from Aintree Hospital, Liverpool (N=303 provided; N=290 with complete data for scoring) as a single-setting validation control.

In the initial stages, a preliminary score comprising 12 independent predictors of death, including care home residency, was devel-

oped; to enable external validation against the ISARIC cohort (which did not include residential data), care home status was excluded as a variable to yield an 11-predictor score. Its ability to discriminate for in-hospital mortality was assessed by the area under the receiver operating characteristic (AUROC). From this score, a condensed version comprising five clinical predictors was developed for prehospital application. Mortality cut-points at each risk level were assessed to define mild, moderate and high risk classes, followed by determination of positive and negative predictive values, as well as sensitivity and specificity thresholds. Model performance was further assessed by calibration using a graphical representation of the HosmerLemeshow 'goodness-of-fit' test to depict agreement between the expected (predicted) and observed (actual) outcome across the entire COVID-19 severity range in both validation cohorts.¹⁷ The summary relationship between the dependent variable (death) and different levels of disease severity in the external ISARIC population was expressed as McFadden's R^2 .¹⁸

Statistical analysis

Categorical variables were expressed as frequency (%), with significance determined by the χ^2 test. Continuous variables were expressed as median (IQR) or mean (SD) and analysed by the *t*-test, Kruskal-Wallis or Mann-Whitney *U* test, as appropriate. ORs were assessed as unadjusted and adjusted values with respect to in-hospital death, the latter determined by multivariate regression with bootstrapping of 1000 resamples. We used this method as internal validation to improve statistical inference by deriving a better estimate of the sampling distribution. Bootstrapping involves randomly drawing repeat samples from the core dataset to calculate SEs and CIs for the final regression analysis. MICE was used to generate valid estimates of randomly missing values in the derivation model. A *p* value of <0.05 was considered statistically significant. All statistical analyses including risk modelling calculations were performed using STATA, V.16 (Stata, Texas, USA).

RESULTS

Baseline demographic and clinical characteristics of the derivation cohort

Nine hundred eighty-three patients (52.5% male) confirmed as SARS-CoV-2 rRT-PCR positive were recruited over the 11-week study period. Five patients remained in hospital at the time of data cut-off on 31 May 2020. The median age of the cohort was 70 (IQR 53–83; range 23–99); median age was lowest in the virtual hospital pathway (53; IQR 43–67) and highest among hospitalised patients who did not receive CPAP (77; IQR 61–86) (*p*<0.001) (online supplemental appendix 1).

The most common comorbidities were hypertension (48.4%), pulmonary disease (30.0%), cardiac disease (26.6%), diabetes mellitus (23.6%), chronic kidney disease (CKD; 20.0%) and dementia (15.4%). Obesity, defined as body mass index (BMI)>30, was present in 24.7% (243) of the cohort and is associated with an unadjusted OR for death of 1.40 (95% CI 1.18 to 2.68, *p*<0.05).

Overall, 294 out of 983 (29.9%) patients died in hospital, the vast majority (97.3%) aged 50 or older. The mortality rates of different age brackets in the cohort (compared with the ISARIC and Aintree validation cohorts) are shown in online supplemental appendix 2. The univariate OR for death increased with rising age, and was 14.86 (95% CI 6.89 to 32.04) for those aged 70–79 and 20.87 (95% CI 9.93 to 43.86) for those aged 80 or older (table 1). When stratified by maximal levels of care, mortality rate was lowest in the VH (1.8%) and highest in the ICU group (62.1%) (*p*<0.001) (online supplemental appendix 3).

White Caucasian ethnicity constituted 77.3% (760/983) of the whole cohort, while Asian, Black and other minor ethnicities (BAME) represented 16.5%, 4.5% and 1.7%, respectively. Overall, white ethnicity was associated with the highest proportion of non-survivors (85.0%); in comparison, patients of Asian (OR 0.57, 95% CI 0.38 to 0.85, *p*<0.01) or black (OR 0.39, 95% CI 0.17 to 0.88, *p*<0.05) background in this cohort had lower ORs for death from COVID-19. The proportion of nonsurvivors within each ethnic group was also highest in white (32.9%), followed by Asian (21.6%), Black (15.9%) and other minority groups (17.6%) (*p*<0.01). Of note, white patients were significantly older by median age (74, IQR 58–85) compared with Asian (57, IQR 46–71; *p*<0.0001) or black (58, IQR 50–72; *p*<0.001).

Table 1 Risk factors of mortality in the derivation cohort (N=983)

	Univariate OR (95%CI)	P value	Likelihood ratio χ^2 of five final predictors	Multivariate OR (95%CI)	P value
Age (years)			2177.27		
<50	1.00			1.00	
50–59	1.30 (1.28 to 7.02)	0.01		2.39 (1.49 to 3.84)	
60–69	6.10 (2.73 to 13.66)	<0.001		2.15 (1.32 to 3.50)	
70–79	14.86 (6.89 to 32.04)	<0.001		7.40 (4.67 to 11.74)	
≥80	20.87 (9.93 to 43.86)	<0.001		10.73 (6.82 to 16.90)	
Male sex (vs female)	1.23 (0.94 to 1.62)	0.137		-	
Ever smoked (vs never smoked)	2.14 (1.52 to 3.01)	<0.001		2.51 (1.91 to 3.29)	
Ethnicity (vs White)					
Asian	0.57 (0.38 to 0.85)	0.006		1.44 (0.71 to 2.94)	
Black	0.39 (0.17 to 0.88)	0.024		0.51 (0.16 to 1.64)	
Symptoms (vs none)					
Breathlessness	0.80 (0.59 to 1.07)	0.135		-	
Fever	0.84 (0.62 to 1.14)	0.26		-	
Cough	0.61 (0.45 to 0.82)	0.001		0.67 (0.41 to 1.09)	
Myalgia	0.41 (0.28 to 0.63)	<0.001		0.79 (0.44 to 1.41)	
Headache	0.51 (0.26 to 0.97)	0.039		1.70 (0.64 to 4.51)	
Clinical parameters					
SpO ₂ (≤92% on air)	3.74 (2.73 to 5.12)	<0.001	31.87	2.69 (1.80 to 4.01)	
Respiratory rate (>24/min)	2.15 (1.63 to 2.84)	<0.001	158.21	2.12 (1.35 to 3.32)	
Systolic BP (≤90 mm Hg)	2.19 (0.96 to 5.03)	0.064		-	
BMI (>30)	1.40 (1.03 to 1.90)	0.033	11.13	2.18 (1.46 to 3.20)	
Frailty (vs not frail, CFS 0–4)					
1 (CFS 5–6)	2.53 (1.73 to 3.70)	<0.001		1.26 (0.82 to 1.96)	
2 (CFS 7–9)	2.56 (1.62 to 4.06)	<0.001		1.28 (0.77 to 2.13)	
Residency in care home (vs own home)	3.14 (2.28 to 4.32)	<0.001		1.38 (0.90 to 2.11)	
Peripheral blood markers					
CRP (>50 mmol/L)	2.40 (1.73 to 3.32)	<0.001		1.42 (0.87 to 2.31)	
Total white cell count					
≤4 × 10 ⁹ /L	0.93 (0.56 to 1.55)	0.79		-	
>11 × 10 ⁹ /L	2.04 (2.09 to 4.15)	<0.001		1.76 (1.18 to 2.61)	
Lymphocytes (<0.7 × 10 ⁹ /L)	1.97 (1.47 to 2.64)	<0.001		1.67 (1.17 to 2.37)	
Chronic kidney disease stage (vs 1, eGFR≥90mL/min/1.73m ²)	1.00	<0.001			
2 eGFR 60–89	2.35 (1.53 to 3.62)	<0.001		0.91 (0.49 to 1.69)	
3 eGFR 30–44 and 45–59	6.09 (3.90 to 9.50)	<0.001		1.46 (0.78 to 2.71)	
4 eGFR 15–29	8.88 (4.82 to 16.34)	<0.001		1.78 (0.82 to 3.85)	
5 eGFR<15	15.73 (6.85 to 36.14)	<0.001		3.82 (1.43 to 10.26)	
CXR ≥4 zones affected, (vs no abnormal zones)	1.89 (1.42 to 2.52)	<0.001		1.73 (1.22 to 2.46)	
Medications (≥5 different)	2.59 (1.93 to 3.48)	<0.001		0.90 (0.53 to 1.52)	
Co-morbidities (vs none)					
Dementia	3.53 (2.46 to 5.08)	<0.001		1.54 (0.97 to 2.44)	
CVA/stroke	3.50 (2.32 to 5.28)	<0.001	44.98	1.92 (1.18 to 3.11)	
Cardiac disease	2.84 (2.13 to 3.80)	<0.001		1.15 (0.70 to 1.91)	
Cancer	2.60 (1.77 to 3.83)	<0.001		1.85 (0.91 to 3.75)	
Hypertension	2.46 (1.85 to 3.26)	<0.001		1.10 (0.65 to 1.88)	
Diabetes mellitus	1.40 (1.02 to 1.91)	0.036		1.00 (0.58 to 1.75)	
Anxiety/psychosis	1.20 (0.84 to 1.71)	0.318		-	
Lung disease	1.15 (0.86 to 1.55)	0.35		-	

Out of the starting 75 variables, those with numerically small ORs or a p value of >0.05 following univariate regression were not included in table 1.

BMI, body mass index; BP, blood pressure; CFS, Clinical Frailty Scale; CRP, c-reactive protein; CVA, cerebrovascular accident; CXR, chest radiograph; eGFR, estimated glomerular filtration rate.

Care home residency (204/983; 20.8%) was more common among non-survivors ($p<0.001$) and was associated with an unadjusted OR for death of 3.14 (95% CI 2.28 to 4.32, $p<0.001$). Based on data from 644 patients aged 65 or older, the univariate OR of frailty for death was 2.52 (95% CI 1.73 to 3.69; $p<0.001$) in those with a group 1 frailty score and 2.56 (95% CI 1.62 to 4.06; $p<0.001$) in those with a group 2 frailty score (table 1).

The median time from symptom onset to presentation for the derivation cohort was 6 days (IQR 2.0–11.0), with no difference between survivors and non-survivors. The four most common reported symptoms were fever (61%), breathlessness (57.9%), cough (52.8%) and myalgia (21.7%). Tachypnoea (respiratory rate>24/minute) and hypoxia (SpO₂≤92% on ambient air) were evident in 35.9% and 31.4% of patients, respectively, and is associated with crude OR for death of 2.15 (95% CI 1.63 to 2.84, $p<0.001$) and 3.74 (95% CI 2.73

to 5.12, $p<0.001$), respectively. C reactive protein>50mg/L was more frequently documented in non-survivors (76.7% vs 58.5%; $p<0.001$) and associated with an unadjusted OR for mortality of 2.40 (95% CI 1.73 to 3.32, $p<0.001$). Lymphopenia was similarly more common in non-survivors (44.7% vs 29.1%; $p<0.001$), with an unadjusted OR for death of 1.97 (95% CI 1.47 to 2.64; $p<0.001$). A baseline chest radiograph (CXR) was available in 91% (895/983) patients; abnormalities in ≥4 radiographic zones were evident in 338 (37.8%) of cases and was associated with increased mortality on univariate analysis (OR 1.89, 95% CI 1.42 to 2.52, $p<0.001$).

Multivariate regression for independent risk factors of mortality

The bootstrapped multivariate regression analysis included the whole derivation cohort of 983 patients comprising 689 (70.1%)

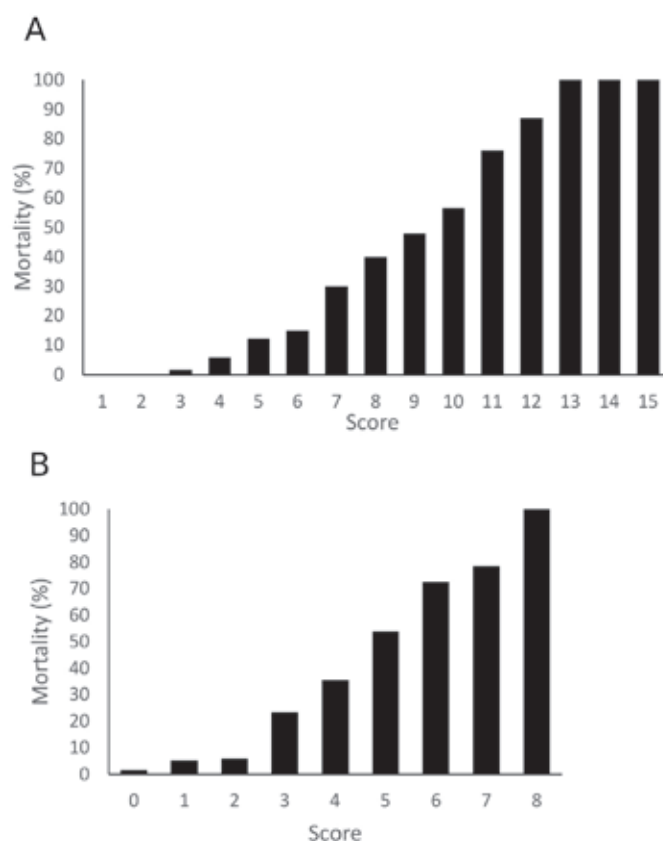


Figure 1 (A) In-patient mortality stratified according to the 11-predictor (SOARS) scores; (B) In-patient mortality stratified according to the 5-predictor (SOARS) scores.

survivors and 294 (29.9%) non-survivors with complete or multiply imputed values for data. Older age, CKD stage 5, baseline hypoxia, elevated BMI, tachypnoea, leucocytosis and a history of stroke were identified as the strongest independent predictors of mortality (table 1). In particular, age ≥ 70 had the highest OR for death over the other individual variables. Following multivariate regression, care home residency no longer independently predicted mortality.

Iterative modelling to construct an 11-predictor and 5-predictor risk prediction scores

ORs and their respective p values from the multivariate logistic regression model were used to identify constituent variables for developing risk prediction scores. We began by constructing an initial 11-predictor score that ranged from 1 to 18 points, using data from 770 patients in the derivation cohort with a complete dataset (online supplemental appendix 4). The lowest score of 1 point reflected the KDIGO (kid-

Table 3 SOARS score (five predictors; range: 0–8 points) for predicting in-hospital COVID-19 death

Cohort	11-predictor	5-predictor (SOARS)	Age ≥ 80	Age 70–79	Age ≥ 50
Derivation	0.84	0.82	0.73	0.76	0.73
Aintree (validation)	0.87	0.80	0.78	0.69	0.57
ISARIC (validation)	0.77	0.74	0.68	0.70	0.63

SOARS, SpO₂, Obesity, Age, Respiratory rate, Stroke history.

ney disease improving global outcomes) categorisation of CKD.¹⁹ In this score, the correlation between increasing COVID-19 severity and in-hospital mortality followed a linear dose–response relationship, particularly between a score of 3 (below which no deaths occurred) and 12 (above which no patient survived), and an accuracy (AUROC) of 0.84 (figure 1). A shorter five-predictor score based solely on clinical parameters and scaled from 0 to 8 points was also developed (table 2; figure 1). This short score, abbreviated as SOARS (SpO₂, Obesity, Age, Respiratory rate, Stroke history), demonstrated an AUROC of 0.82 and was retained for further evaluation as a practical prehospital risk stratification tool.

External validation and performance of the SOARS score

The performance metrics of the long 11-predictor and 5-predictor (SOARS) scores were assessed by their ability to discriminate for in-hospital mortality against both the ISARIC and Aintree validation cohorts (table 3). The longer score showed higher discrimination in the Aintree (AUROC 0.87) than the ISARIC cohort (0.77). The SOARS score had a slightly lower AUROC against both cohorts, namely, 0.80 (Aintree) and 0.74 (ISARIC). In comparison, the performance of other scores based solely on different cut-offs of age were associated with inferior discriminatory ability. Comparison of some of the main population parameters between the derivation and both external cohorts is shown in online supplemental appendix 5.

The mortality rate at each level of the SOARS score in the derivation and both validation cohorts are shown in table 4. For increased applicability, the SOARS score results were further categorised into three risk classes: low (SOARS 0–1), moderate (SOARS 2) and high (SOARS ≥ 3) (table 5). Between 2.3% and 3.2% of patients scoring 0 or 1 (low risk) in each of the three cohorts died due to COVID-19, whereas 2.3%–6.3% of those scoring 2 (moderate risk) failed to survive to discharge. Overall, 9 out of every 10 deaths in each of the derivation and validation cohorts had a SOARS score of 3 or greater. Within this broad range of increasing scores, the highest proportion of deaths was encountered at SOARS score 4 in both the derivation cohort and the ISARIC validation cohort (28.3% and 34.5%, respectively) and at SOARS score 5 in the Aintree validation cohort (30.9%).

Sensitivity thresholds calculated for the larger validation (ISARIC) cohort showed that the low risk class (SOARS 0–1) comprised 16.6% of patients with an in-hospital mortality of 5.4%, sensitivity of 97% and negative predictive value (NPV) of 94.6% (table 6). By comparison, 13.2% of patients were classified as moderate risk; their mortality rate was 14.5%. When combining the low and moderate risk groups (SOARS 0–2), the sensitivity reduces to 90.7% and the NPV to 90.5%. The high-risk group comprised 70.2% of the validation cohort who scored across a wide range of SOARS (scores 3–8). The specificity for a prediction of death was thus more variable, from 58.2% for a SOARS score of 3 to 99.8% at the other end of the scale when the SOARS score was 7. Only one patient scored 8; they survived to discharge.

Reliability of the risk estimates in the ISARIC cohort, modelled as calibration or goodness-of-fit between expected (predicted) and observed outcomes using SOARS, showed a calibration slope of 0.70, calibration-in-the-large (CiTL) 0.02 and an expected-to-observed (E:O) ratio of 0.990 (figure 2). Calibration was slightly improved in the Aintree cohort with a slope of 0.80, CiTL –0.16 and E:O ratio of 1.06. A LOWESS (locally weighted scatterplot smoothing algorithm) curve was generated to show differences between these outcomes in both cohorts. The plot characteristics suggested that the model, while demonstrating good concordance, had greater predictive accuracy in low-risk to moderate-risk patients whose predicted probability of mortality was under 40%. Conversely, overestimation of mortality risk was evident in patients in the high-risk group.

Table 2 SOARS score (five predictors; range: 0–8 points) for predicting in-hospital COVID-19 death

Predictor	Points
SpO ₂	
>92% on air	0
≤92% on air	1
Obesity (BMI >30)	
Absent	0
Present	1
Age (years)	
<50	0
50–59	1
60–69	2
70–79	3
≥80	4
Respiratory rate	
≤24/min	0
>24/min	1
Stroke/CVA	
Absent	0
Present	1

CVA, cerebrovascular accident; SOARS, SpO₂, Obesity, Age, Respiratory rate, Stroke history.

Table 4 Risk factors of mortality in the derivation cohort (N=983)

Score	Derivation cohort (N=821; deaths=258)			Aintree validation cohort (N=290; deaths=94)			ISARIC validation cohort (N=14231; deaths=4319)		
	No of deaths (n/N)	Mortality rate (%)	Proportion of deaths (%)	No of deaths (n/N)	Mortality rate (%)	Proportion of deaths (%)	No of deaths (n/N)	Mortality rate (%)	Proportion of deaths (%)
0	1/69	1.4%	0.4%	1/12	8.3%	1.1%	34/833	4.1%	0.8%
1	5/94	5.3%	1.9%	2/23	8.7%	2.1%	94/1529	6.1%	2.2%
2	6/102	5.9%	2.3%	3/37	8.1%	3.2%	273/1879	14.5%	6.3%
3	29/124	23.4%	11.2%	11/60	18.3%	11.7%	650/2577	25.2%	15.1%
4	73/206	35.4%	28.3%	24/72	33.3%	25.5%	1490/4013	37.1%	34.5%
5	63/117	53.9%	24.4%	29/48	60.4%	30.9%	1170/2402	48.7%	27.1%
6	58/80	72.5%	22.6%	16/30	53.3%	17.0%	571/939	60.8%	13.2%
7	22/28	78.6%	8.5%	6/6	100.0%	6.4%	36/58	62.1%	0.8%
8	1/1	100.0%	0.4%	2/2	100.0%	2.1%	0/1	0.0%	0.0%

Out of the starting 75 variables, those with numerically small ORs or a p value of >0.05 following univariate regression were not included in table 1.

BMI, body mass index; BP, blood pressure; CFS, Clinical Frailty Scale; CRP, c-reactive protein; CVA, cerebrovascular accident; CXR, chest radiograph; eGFR, estimated glomerular filtration rate.

DISCUSSION

We show that prognostic evaluation of a small panel of baseline clinical and demographic characteristics of patients with COVID-19 enables their subsequent risk of in-hospital death to with COVID-19-suspected individuals. This role is not met by currently available prediction tools that rely on laboratory measurements. The SOARS score discriminated well for COVID-19 mortality and its simplicity obviated the need for complex calculations. It also retained good predictive accuracy in two external validation cohorts, with performance metrics that were primarily reflected in its high negative predictive values for mortality among patients with the lowest risk scores (0 or 1). This characteristic is consistent with a high accuracy for predicting a non-fatal outcome in its key target group, namely, individuals with milder COVID-19.

Patients who score SOARS 0 or 1 could be discharged home with advice to re-establish urgent contact if their symptoms worsened. Patients stratified as moderate risk (SOARS 2) could be virtually monitored with a predefined plan for care escalation if specific thresholds relating to deteriorating symptoms or self-recorded SpO₂ were triggered. Patients in the high-risk class (score ≥3) are highly likely to be symptomatic and would, in all probability, be referred directly to the ED for hospital-based management. Thus, the target individuals for the SOARS score are those with a low or moderate risk of COVID-19 mortality.

Our data concur with other reports that advanced age is the strongest predictor of death from COVID-19.^{20–23} The increase in mortality in patients within the derivation cohort who were in or beyond their seventh decade of life was reflected in the magnitude of their respective adjusted ORs for in-hospital death,

namely, 7.4 (aged 70–79) and 10.7 (aged ≥80). Even so, predictive models generated with age as the lone variable showed poorer discriminatory ability than the SOARS score.

The early development of physiological abnormalities in COVID-19 does not always result in timely clinical presentation. In our study, two measures of acute physiological perturbation proved to be important predictors of COVID-19 mortality: hypoxia and tachypnoea. Although persistent hypoxia is more common in non-survivors of COVID-19, its relationship with tachypnoea remains incompletely understood.^{24,25} Fewer than half of patients with COVID-19 who present to hospital with decreased oxygen saturation report experiencing subjective breathlessness.^{26–29} One reason for this observation might be the so-called ‘silent hypoxia’ where a blunted symptomatic perception of the effects of hypoxaemia is apparent even when low arterial oxygen tension is evident.³⁰ This phenomenon may be responsible for delays in seeking clinical attention. Such danger could be mitigated by accurate risk assessment including the measurement and tracking of SpO₂ in patients who are deemed to not require immediate hospitalisation. The absence of oxygen determination in the CURB-65 score has been cited as limiting its utility in stratifying patients with COVID-19 for outpatient management.³¹

The SOARS score was constructed with data from hospitalised patients as the very low adverse event rate among non-admitted cases (eg, in the VH pathway) curtailed the development of a prognostic tool. This issue has previously been highlighted in the context of CRB-65 where low event rates in community studies of pneumonia made predictive inferences difficult to conclude.⁹ Other scores that have been used in COVID-19 studies have either not been designed for this disease or have relied heavily on laboratory-measured data.^{32–35}

Table 5 COVID-19 mortality risk stratification based on SOARS score

Risk class score level	Derivation cohort (N=821; deaths=258)		Aintree validation cohort (N=290; deaths=94)		ISARIC validation cohort (N=14231; deaths=4319)	
	Mortality by risk class (%)	Proportion of deaths by risk class (%)	Mortality by risk class (%)	Proportion of deaths by risk class (%)	Mortality by risk class (%)	Proportion of deaths by risk class (%)
Low (0–1)	6/163 (3.7%)	6/258 (2.3%)	3/35 (8.6%)	3/94 (3.2%)	128/2362 (5.4%)	128/4319 (3.0%)
Moderate (2)	6/102 (5.9%)	6/258 (2.3%)	3/37 (8.1%)	3/94 (3.2%)	273/1879 (14.5%)	273/4319 (6.3%)
High (≥3)	246/556 (44.2%)	246/258 (95.3%)	88/218 (40.4%)	88/94 (93.6%)	3917/9990 (39.2%)	3917/4319 (90.7%)

Out of the starting 75 variables, those with numerically small ORs or a p value of >0.05 following univariate regression were not included in table 1.

BMI, body mass index; BP, blood pressure; CFS, Clinical Frailty Scale; CRP, c-reactive protein; CVA, cerebrovascular accident; CXR, chest radiograph; eGFR, estimated glomerular filtration rate.

Table 6 Sensitivity analysis of the SOARS score for predicting mortality in the ISARIC validation cohort

Score cut-off	Patients N (% of total)	True positive	True negative	False positive	False negative	Sensitivity	Specificity	PPV	NPV	Cumulative mortality (%)
>0	833 (5.9%)	4284	799	9114	34	99.2%	8.1%	32.0%	95.9%	34/833 (4.1%)
>1	2362 (16.6%)	4190	2234	7679	128	97.0%	22.5%	35.3%	94.6%	128/2362 (5.4%)
>2	4241 (29.8%)	3917	3840	6073	401	90.7%	38.7%	39.2%	90.5%	401/4241 (9.5%)
>3	6818 (47.9%)	3267	5767	4146	1051	75.7%	58.2%	44.1%	84.6%	1051/6818 (15.4%)
>4	10 831 (76.1%)	1777	8290	1623	2541	41.2%	83.6%	52.3%	76.5%	2541/10 831 (23.5%)
>5	13 233 (93.0%)	607	9522	391	3711	14.1%	96.1%	60.8%	72.0%	3711/13 233 (28.0%)
>6	14 172 (99.6%)	36	9890	23	4282	0.8%	99.8%	61.0%	69.8%	4282/14 172 (30.2%)
>7	14 231 (100.0%)	0	9912	1	4318	0.0%	100.0%	0.0%	69.7%	4318/14 231 (30.3%)

Out of the starting 75 variables, those with numerically small ORs or a p value of >0.05 following univariate regression were not included in table 1.

BMI, body mass index; BP, blood pressure; CFS, Clinical Frailty Scale; CRP, c-reactive protein; CVA, cerebrovascular accident; CXR, chest radiograph; eGFR, estimated glomerular filtration rate.

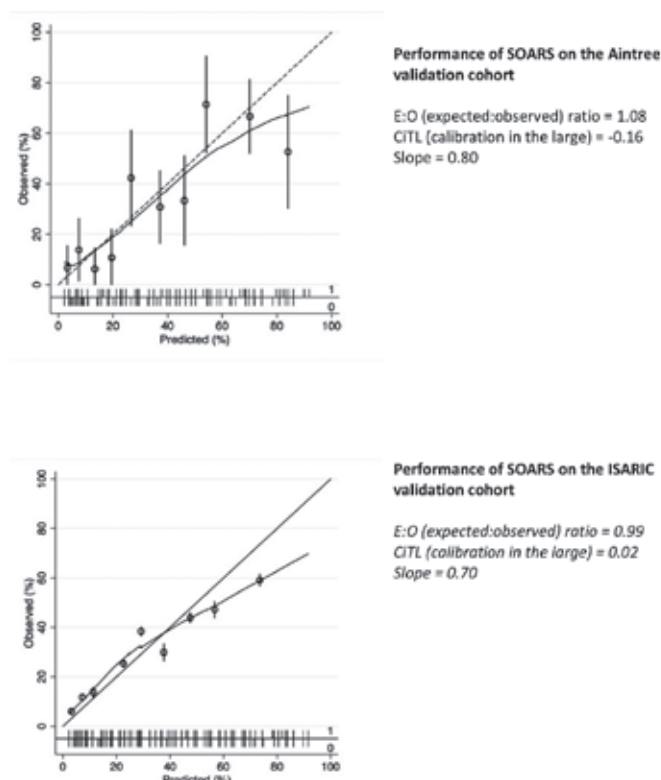


Figure 2 Calibration accuracy of the SOARS (SpO₂, Obesity, Age, Respiratory rate, Stroke history) score on external validation cohorts.

Our multivariate regression model was bootstrapped to reduce overfitting but not penalised prior to external validation. In common with other severity scores for COVID-19, we dichotomised several continuous data parameters which may have potentially obscured non-linear effects between predictors and outcome, contributing to the difference in AUROC values between our derivation and validation cohorts and between both validation cohorts.^{11 32 34} Other prediction systems, notably CURB-65 and the Pneumonia Severity Index for pneumonia similarly categorise some of their score parameters.^{10 36} We also used in-hospital mortality as an unambiguous disease-related primary outcome rather than 30-day or 60-day mortality. The better performance of the SOARS score in the smaller Aintree validation cohort compared with the much larger ISARIC cohort may have been due to its more homogeneous case-mix. This comparison suggested that, on balance, the simplicity of a prehospital risk prediction tool, provided it retained acceptable accuracy, may outweigh any minor diminution of its performance arising from improved practicality.

Other limitations in the study include the occurrence of missing information despite prospective data collection. The use of multiple imputation to estimate missing values for multivariate regression and the availability of nearly 85% of observations for constructing the risk stratification rule helped to mitigate against underestimating their role. The modest sample size of our derivation cohort was dictated by the incident caseload during the pandemic. However, selective sampling of the pandemic timeline was avoided by including all COVID-19 cases from the initial rise to the subsequent decline in new case numbers over the 11-week study period. Finally, reduced score calibration at the high-risk end suggests that SOARS may overestimate the probability of death in the highest risk cases. However, the principal objective of this score was to enhance frontline decision-making in patients with a low predicted risk of mortality at a time when demand for in-patient resources is likely to be high.

In summary, prognostication using the SOARS score can be undertaken concomitantly with SARS-CoV-2 diagnostic testing to inform clinical triaging, including decisions about the placement of the patient for ongoing care. Analysis of the ISARIC validation cohort in this study showed that between 16.6% and 29.8% (those scoring up to SOARS 1 or 2, respectively) could potentially have avoided admission provided a safe alternative to hospitalisation was in place. Prospective studies of SOARS implementation will enable the score to be calibrated against other independent cohorts of

patients with COVID-19 to examine its performance under conditions that may be unique to different localities. Such an opportunity may soon present itself if SARS-CoV-2 transmission continues to increase in the UK and beyond.

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Data availability statement Data are available upon reasonable request. Deidentified participant data may be requested from the corresponding author following publication of the study (ORCID identifier: 0000-0001-7845-0173). This article is made freely available for use in accordance with BMJ's website terms and conditions for the duration of the covid-19 pandemic or until otherwise determined by BMJ. You may use, download and print the article for any lawful, non-commercial purpose (including text and data mining) provided that all copyright notices and trade marks are retained.

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Insuficiência de vitamina D e risco de pneumonias bacterianas: Estudos de aleatorização mendeliana em duas coortes de base populacional

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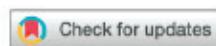
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RESUMO

Contexto A vitamina D pode regular o sistema imunitário inato e ensaios aleatórios controlados sugerem um efeito benéfico do suplemento de vitamina D contra infecções agudas do trato respiratório. Utilizando uma abordagem de aleatorização mendeliana, testamos a hipótese de que níveis baixos de 25-hidroxivitamina D estão associados a um risco acrescido de pneumonia bacteriana em análises observacionais e genéticas.

Métodos Determinamos o genótipo de 116 335 dinamarqueses caucasianos com idades entre os 20 e 100 anos selecionados aleatoriamente do *Copenhagen City Heart Study* e do *Copenhagen General Population Study* para variantes genéticas de insuficiência de 25-hidroxivitamina D plasmática em torno de CYP2R1 (rs117913124, rs12794714 e rs10741657), DHCR7 (rs7944926 e rs11234027), GEMIN2 (rs2277458) e HAL (rs3819817). A informação sobre 25-hidroxivitamina D plasmática estava disponível em 35 833 indivíduos. Os indivíduos foram seguidos entre 1981 e 2018 para diagnósticos hospitalares de pneumonia bacteriana.

Resultados Durante 38 anos de seguimento, observamos 6342 pneumonias bacterianas em análises observacionais e 13 916 em análises genéticas. Nas análises observacionais, o HR ajustado para multivariáveis para pneumonias bacterianas foi de 1,27 (95% IC: 1,16 a 1,40) para indivíduos com 25-hidroxivitamina D <25 nmol/L em comparação com indivíduos com ≥25 nmol/L. Nas análises genéticas, o OR para pneumonia bacteriana por 10 nmol/L de níveis plasmáticos reduzidos de 25-hidroxivitamina D foi de 1,12 (95% IC: 1,02 a 1,23) no rácio de Wald, 1,12 (95% IC: 1,04 a 1,20) na variância inversa ponderada, 1,63 (95% IC: 0,96 a 2,78) na aleatorização mendeliana de Egger e 1,15 (95% IC: 1,05 a 1,26) na análise da variável instrumental da mediana ponderada. Esta associação foi mais notória para as variantes genéticas em torno de CYP2R1. Não se verificou qualquer evidência observacional ou genética que sustente que a 25-hidroxivitamina D esteja associada ao risco de infecções do trato urinário, infecções cutâneas, septicemia ou gastroenterite, que foram utilizadas como resultados de controlo negativo.

Conclusões A insuficiência de vitamina D está associada, em termos observacionais e genéticos, a um risco acrescido de pneumonias bacterianas.

INTRODUCTION

The proposed role of vitamin D in human health has been extended well beyond calcium and bone homeostasis to now include regulation of the innate immune system.^{1,2} Accordingly, observational studies have found that low plasma 25-hydroxyvitamin D concentrations are associated with increased risk of respiratory tract infections in adults, children and infants.^{3–9} Nevertheless, randomised controlled trials investigating effect of vitamin D supplementation against respiratory tract infections have found conflicting results.^{10–23}

Key messages

What is the key question?

► This study was designed to answer whether low plasma vitamin D is a causal factor in bacterial pneumonias by using a Mendelian randomisation approach.

What is the bottom line?

► By using a Mendelian randomisation approach in two population-based cohorts with >110 000 individuals and up to 38 years of follow-up, low plasma vitamin D is associated observationally and genetically with increased risk of bacterial pneumonias, but not with risk of urinary tract infections, skin infections, sepsis or gastroenteritis.

Why read on?

► This is the first study that investigates the causal association of vitamin D with bacterial pneumonias alone using genetic evidence.

However, a systematic review and meta-analysis comprising 11 321 participants and including all randomised controlled trials with vitamin D supplementation of any duration against acute respiratory tract infections showed a beneficial effect.²⁴ To our knowledge, no study has attempted to investigate the causal association of vitamin D with bacterial pneumonias alone using genetic evidence.

A Mendelian randomisation analysis takes advantage of natural randomisation and uses genetic variants as proxies of modifiable exposures.²⁵ Since alleles are randomly distributed at conception, genetic variants should not be associated with potential confounders, and since genes are present at birth, genetic analyses are not susceptible to reverse causation. Thus, causal inferences can be made using such a study design akin to randomised controlled trials. Indeed, genetic variants have been identified that specifically lead to lifelong lower plasma 25-hydroxyvitamin D concentrations,^{26–29} providing an ideal framework to assess low 25-hydroxyvitamin D as a causal risk factor for bacterial pneumonias. In such a Mendelian randomisation framework, instruments or genetic variants known to be associated with plasma 25-hydroxyvitamin D are tested for their association with bacterial pneumonia. From this, it may be possible to show that 25-hydroxyvitamin D is causal of bacterial pneumonia under a number of key assumptions. The assumptions are that the genetic variants are associated with 25-hydroxyvitamin D, are only associated with

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bacterial pneumonia through 25-hydroxyvitamin D and are independent of confounders of the association of plasma 25-hydroxyvitamin D with bacterial pneumonia.

By using a Mendelian randomisation approach, we tested the hypothesis that low 25-hydroxyvitamin D is associated with increased risk of bacterial pneumonia in observational and genetic analyses. For this purpose, we used two population-based cohorts with >110 000 individuals and up to 38 years of follow-up.

MATERIALS AND METHODOLOGY

Study populations

The Copenhagen City Heart Study is a population-based prospective cohort study initiated in 1976 to 1978, with follow-up examinations in 1981 to 1983, 1991 to 1994 and 2001 to 2003.30 All individuals in Denmark are assigned a unique identification number at birth or immigration (Central Person Registration number) and recorded in the national Danish Civil Registration System. Individuals aged 20 to 100 were randomly selected and invited from the national Danish Civil Registration System to reflect the white adult general population of Danish descent (an individual is registered as of Danish descent in the national Danish Civil Registration System if the person and both parents are born in Denmark and have Danish citizenship). At study entry, all individuals completed a questionnaire, underwent a physical examination and gave blood for biochemical and genetic analyses (1991 to 1994 and 2001 to 2003 examinations only). In observational analyses, we included 10 272 individuals with plasma 25-hydroxyvitamin D measurements from the 1981 to 1983 examination. In genetic analyses, we included 9536 individuals from the 1991 to 1994 and/or 2001 to 2003 examinations with information on all genotypes. Of these, 5597 had information on both plasma 25-hydroxyvitamin D measurements and all genotypes.

The Copenhagen General Population Study is a population-based prospective cohort study initiated in 2003 with ongoing enrolment, recruited and examined as the Copenhagen City Heart Study but from different parts of Copenhagen.³⁰ In observational analyses, we included 25 561 individuals, with plasma 25-hydroxyvitamin D measurements. In genetic analyses, we included 106 799 individuals with information on all genotypes. Of these, 25 410 had information on both plasma 25-hydroxyvitamin D measurements and all genotypes.

No individual appeared in more than one study. For the Copenhagen City Heart Study, the response-rate was 72% in the 1976 to 1978 examination, 63% in the 1981 to 1983 examination, 61% in the 1991 to 1994 examination and 50% in the 2001 to 2003 examination. Non-responders versus responders in the 1976 to 1978 examination were more often men (52% vs 46%) and slightly older (median age: 55 vs 54 years). Similar differences could be observed between non-responders and responders for the subsequent examinations: 53% vs 45% and 61 vs 58 years in the 1981 to 1983 examination, 45% vs 44% and 63 vs 61 years in the 1991 to 1994 examination and 43% vs 43% and 67 vs 62 years in the 2001 to 2003 examination, respectively. For the Copenhagen General Population Study recruiting in 2003 to 2015, response rate was 43%. Non-responders versus responders were more often men (48% vs 45%) and slightly younger (median age: 56 vs 58).

Plasma 25-hydroxyvitamin D measurements

Measurements of plasma 25-hydroxyvitamin D using the DiaSorin Liaison 25(OH)vitamin D Total Assay, a direct competitive chemiluminescence immunoassay, were conducted blind to information on outcome and genotypes.³⁰ For the Copenhagen City Heart Study, plasma samples were collected in 1981 to 1983 (n=10 272) and stored at -20°C for approximately 30 years before measurement. For the Copenhagen General Population Study, plasma samples were either collected in 2004 to 2005 (n=12 506) and stored at -80°C for approximately 5 years before measurement or in 2009 to 2011 (n=13 055) for measurement on fresh samples. Assay precision was tested daily, while assay accuracy was tested monthly

using an external quality control programme (Vitamin D External Quality Assessment Scheme (DEQAS)). The inter-assay coefficient of variance was 10% for low concentration control (~40nmol/L) and 8% for high concentration control (~135nmol/L).

Genotypes

Genotyping using TaqMan assays was conducted blind to information on outcome and plasma 25-hydroxyvitamin D concentrations.³⁰ Specific genotypes for plasma 25-hydroxyvitamin D concentration were selected as those having the strongest and largest association in genome-wide association studies,²⁶⁻²⁹ we chose genetic variants around CYP2R1 (rs12794714 and rs10741657) and DHCR7 (rs7944926 and rs11234027), as they are expected to influence biologically active plasma 25-hydroxyvitamin D concentrations via either synthesis of previtamin D from 7-dehydrocholesterol in the skin or conversion of vitamin D to 25-hydroxyvitamin D in the liver. We on purpose did not genotype for variants in the vitamin D binding protein, as such variants will influence the amount of 25-hydroxyvitamin D transported in plasma rather than differences in biologically active plasma 25-hydroxyvitamin D. Recently, three additional genotypes for plasma 25-hydroxyvitamin D concentration were identified in genome-wide association studies; a rare genetic variant located around CYP2R1 (rs117913124) and two frequent genetic variants around GEMIN2 (rs2277458) and HAL (rs3819817),²⁷⁻²⁸ which we also genotyped. Call rates were >99% after two reruns.

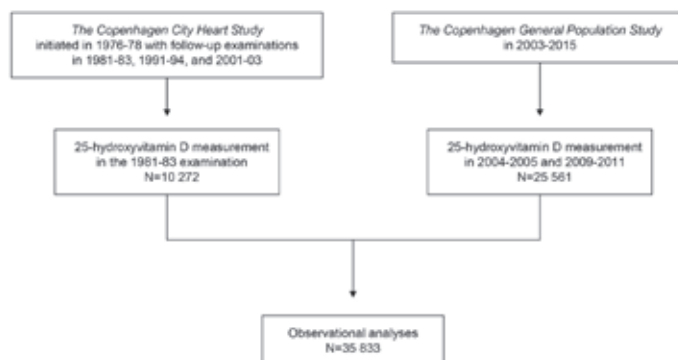
Bacterial pneumonias

Bacterial pneumonias (International Classification of Diseases (ICD)-8: 481xx to 486xx and ICD-10: A481, J13 to J16, J170, J18) were defined as acute emergency department visits and hospital admissions with the mentioned primary diagnosis. Information was obtained from the national Danish Patient Registry, which records all public and private hospital contacts in Denmark, recorded from baseline until 13 December 2018. Diagnoses recorded in the registry are made by medical doctors according to national law. Denmark used the ICD-8 until 1 January 1994 and proceeded directly to ICD-10 after this date. As negative control outcomes, we included urinary tract infections, skin infections, sepsis and gastroenteritis.³¹ By using the unique Central Person Registration number provided to everyone in Denmark at birth or immigration and linking it with the national Danish Patient Registry, no person was lost to follow-up, and individuals who emigrated were censored at the date of emigration (n=137 in observational analyses and n=538 in genetic analyses).

Potential confounders

Body mass index (BMI) was calculated as measured weight divided by measured height squared (kg/m²). Smoking status was defined as never, former or current smoking. Cumulated tobacco consumption was calculated in pack-years based on information on age at smoking initiation and cessation (or for current smokers until age at baseline examination), duration of tobacco consumption and amount of consumed tobacco in form of number of daily consumed cigarettes, cheroots and cigars and grams of weekly consumed pipe tobacco: a pack-year was defined as 20 cigarettes or equivalent smoked daily for a year. Alcohol consumption was self-reported in units per week (1 unit=12g). Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine measured using standard hospital assays. Chronic disease at baseline included diabetes mellitus, ischaemic heart disease, chronic obstructive pulmonary disease (COPD) and cancer. Diabetes at baseline was based on self-report, nonfasting plasma glucose >11mmol/L, use of antidiabetic medication and/or previous inpatient/outpatient hospital contacts due to diabetes (ICD-8: 249 to 250 and ICD-10: E10 to E14), obtained from the national Danish Patient Registry. Ischaemic heart disease (ICD-8: 410 to 414 and ICD-10: I20 to I25) and COPD (ICD-8: 491 to 492 and ICD-10: J41 to J44) at baseline were based on inpatient/outpatient hospi-

Observational analyses flowchart



Genetic analyses flowchart

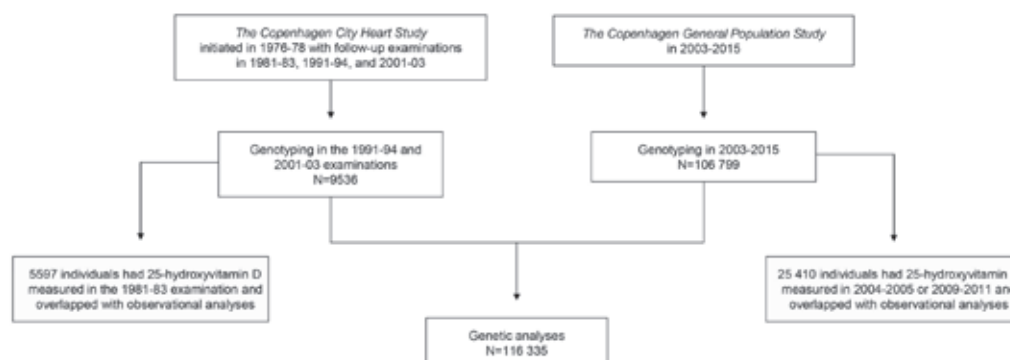


Figure 1 Flowchart depicting samples used in observational and genetic analyses.

tal contacts, obtained from the national Danish Patient Registry. Cancer at baseline, excluding non-melanoma skin cancer cases, was obtained from the national Danish Cancer Registry.

Statistical analyses

Deviation from Hardy-Weinberg equilibrium was investigated using the χ^2 -tests; deviation may suggest genotyping or population sampling errors, but we observed none. Associations with potential confounders were investigated using linear and logistic regression models, as appropriate. Associations between the genotypes and plasma 25-hydroxyvitamin D were investigated using linear regression models; we evaluated the strength of the genotypes as instruments by examining the F-statistic and R^2 : a high F and/or R^2 indicates sufficient strength to ensure statistical reliability of the risk estimates.³² Kaplan-Meier estimator was used to determine cumulative prevalence of bacterial pneumonias with log-rank test. Cox proportional hazard models were used to determine risk of bacterial pneumonias. Multiple failure-time analysis was carried out to investigate risk of recurrent events using the

method proposed by Andersen and Gill.³³ Wald's test was used to assess interaction on risk of bacterial pneumonias. We used age as the underlying timescale (=age adjusted) with left truncation (=delayed entry) at study examination. Observational analyses were adjusted for age, sex, seasonal variation of 25-hydroxyvitamin D (by adjusting for month and year of blood sampling), birth year, cohort, BMI, smoking status, cumulative tobacco consumption, alcohol consumption, eGFR and chronic disease at baseline (diabetes, ischaemic heart disease, COPD and cancer). Genetic analyses were adjusted for age, sex, birth year and cohort, as genetic analyses generally are not prone to confounding. In order to estimate the causal effect of plasma 25-hydroxyvitamin D on bacterial pneumonia, we used instrumental variable analysis with individual level data, accounting for partial overlap and methods designed to be robust to some violations of the assumptions for Mendelian randomisation analyses, that is, inverse-variance weighted, MR-Egger and weighted median estimators.^{34 35} All instrumental variable analyses were carried out using single events, that is, first event, as methodology for investigating recurrent events has

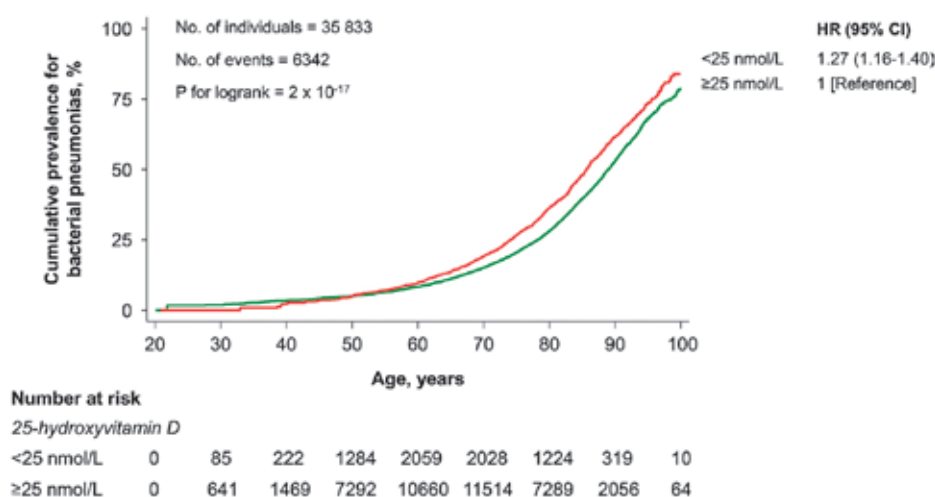
Table 1 Potential confounders in observational and genetic analyses in individuals in the general population

	Individuals in observational analyses (n=35 833)	Association with 5-hydroxyvitamin D concentration, P value*	Individuals in genetic analyses (n=116 335)	Association with genetic risk score, P value*
Age – years	58.1 (48.8 to 66.6)	4×10 ⁻¹³	58.3 (48.1 to 67.7)	0.23
Men – no. (%)	16 076 (45)	5×10 ⁻²⁸	52 339 (45)	0.26
BMI – kg/m ²	25.4 (23.0 to 28.2)	2×10 ⁻¹¹⁶	25.5 (23.1 to 28.4)	0.91
Active smokers – no. (%)	11 230 (31)	5×10 ⁻²⁸⁶	22 787 (20)	0.16
Cumulative tobacco consumption – pack-years†	20.0 (9.0 to 35.0)	2×10 ⁻⁹⁸	16.5 (6.3 to 31.0)	0.96
Alcohol – units/day	1.0 (0.3 to 2.0)	0.002	1.1 (0.4 to 2.1)	0.72
eGFR – mL/min	77 (65 to 89)	9×10 ⁻⁴³	80 (69 to 91)	0.68
Chronic disease at baseline				
Diabetes – no. (%)	1507 (4)	2×10 ⁻¹⁰	4945 (4)	0.30
Ischaemic heart disease	1925 (5)	0.001	6616 (6)	0.79
COPD – no. (%)	725 (2)	0.02	2405 (2)	0.39
Cancer – no. (%)	2091 (6)	2×10 ⁻⁶	7798 (7)	0.27

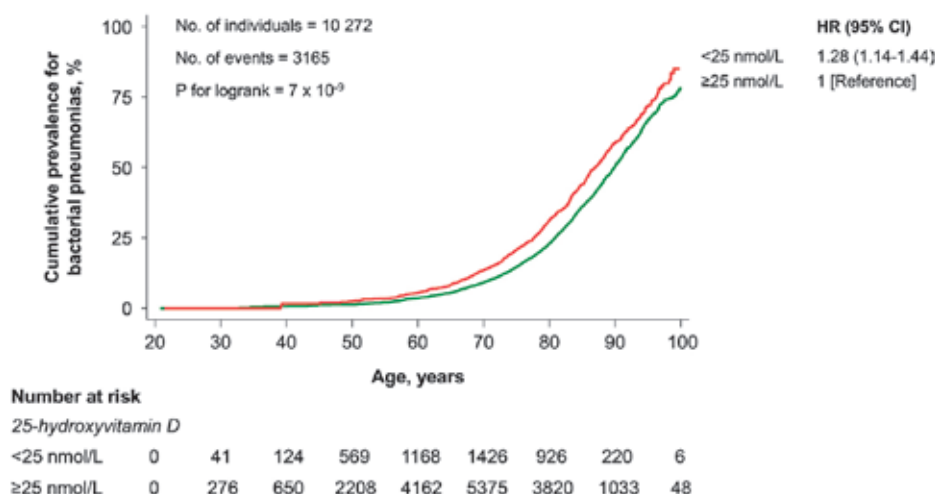
Out of the starting 75 variables, those with numerically small ORs or a p value of >0.05 following univariate regression were not included in table 1.

BMI, body mass index; BP, blood pressure; CFS, Clinical Frailty Scale; CRP, c-reactive protein; CVA, cerebrovascular accident; CXR, chest radiograph; eGFR, estimated glomerular filtration rate.

The Copenhagen City Heart Study and Copenhagen General Population Study



The Copenhagen City Heart Study



The Copenhagen General Population Study

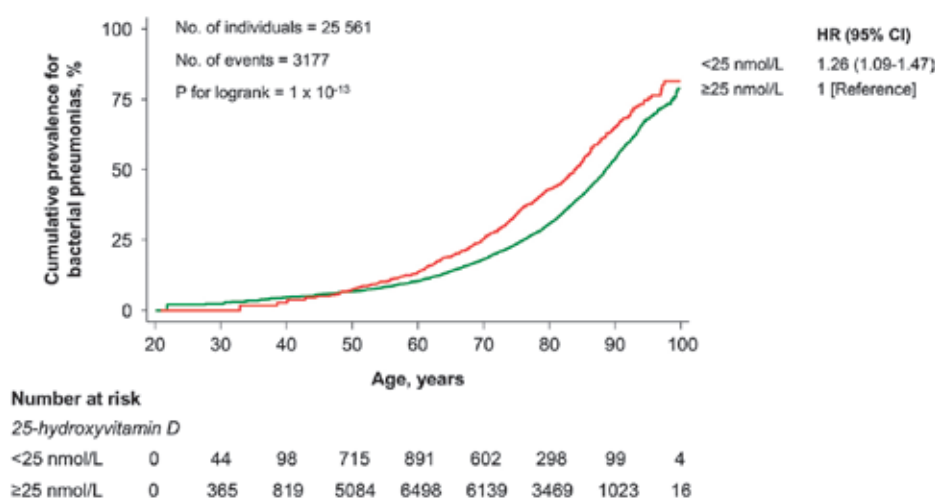


Figure 2 Cumulative prevalence and risk of bacterial pneumonias according to plasma 25-hydroxyvitamin D concentration in individuals in the general population. Cumulative prevalence from Kaplan-Meier estimator, and risk estimates obtained from Cox regression models with multiple failure-time analysis according to Andersen and Gill adjusted for age (as timescale), sex, seasonal variation of 25-hydroxyvitamin D, birth year, cohort, body mass index, smoking status, cumulative tobacco consumption, alcohol consumption, estimated glomerular filtration rate, chronic disease at baseline (diabetes, ischaemic heart disease, chronic obstructive pulmonary disease and cancer).

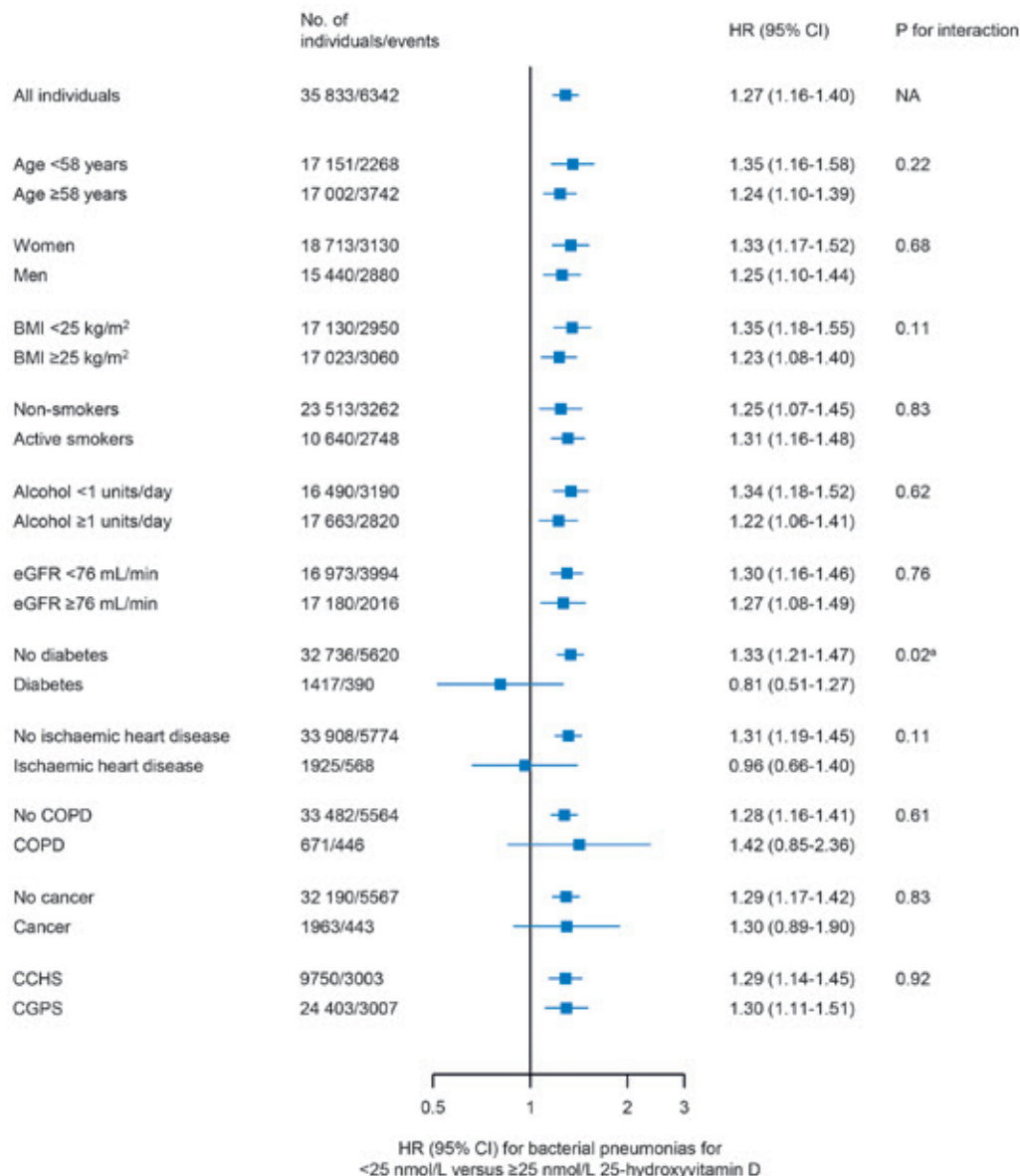


Figure 3 Risk of bacterial pneumonias in different subgroups according to plasma 25-hydroxyvitamin D concentration in individuals in the general population. Risk estimates obtained from Cox regression models with multiple failure-time analysis according to Andersen and Gill adjusted for age (as timescale), sex, seasonal variation of 25-hydroxyvitamin D, birth year, cohort, body mass index (BMI), smoking status, cumulative tobacco consumption, alcohol consumption, estimated glomerular filtration rate (eGFR), chronic disease at baseline (diabetes, ischaemic heart disease, chronic obstructive pulmonary disease (COPD) and cancer), as appropriate. P for interaction obtained from Wald's test. Number of individuals vary slightly in the subgroup analyses due to availability of data. Chosen cut-off values for age, BMI, alcohol and eGFR represent median values. Based on the Copenhagen City Heart Study (CCHS) and Copenhagen General Population Study (CGPS) if not otherwise stated. Risk estimates for the CCHS and CGPS vary slightly from figure 1, as complete case analysis has been used here.

not been developed yet. The causal effect was estimated by including genetic variants that were not prone to weak instrument bias (sufficiently high F-statistic and R^2) in accordance with previous recommendations.³²⁻³⁶ However, instrumental variable analysis was repeated by also including all genetic variants. In a sensitivity analysis, we investigated the associations in the UK Biobank through GeneAtlas.³⁷⁻³⁸ However, UK Biobank had a different outcome definition (influenza and all pneumonia combined) from the one used in the present study, as bacterial pneumonia alone was not available. In another sensitivity analysis, we combined genetic variants around CYP2R1 to create unweighted and weighted genetic risk score, since most of the association seemed to be driven through these genotypes; only internal weighted risk scores could be calculated as genome-wide association studies did not report results necessary to calculate externally weighted risk score.²⁶⁻²⁹ Since information on potential confounders was 99.9% complete, we performed multivariate imputation using chained equations to fill out the missing values in the observational analyses; however, results were similar without the use of imputation. We used Stata/SE 13.1 for Windows.

RESULTS

We had 35 833 individuals in observational analyses and 116 335 in genetic analyses (figure 1 and table 1). Plasma 25-hydroxyvitamin D concentrations were associated with all potential confounders (online supplemental table S1). In contrast, the genotypes for 25-hydroxyvitamin D were not associated with any potential confounders (online supplemental table S2) but as expected associated with plasma 25-hydroxyvitamin D concentrations in a dose-dependent manner (online supplemental figure S1), illustrating that such genotypes can be used as largely unconfounded instruments to assess the association of genetically determined low 25-hydroxyvitamin D with risk of bacterial pneumonias by using a Mendelian randomisation approach. Among the genotypes, GEMIN2 rs2277458 and HAL rs3819817 had F-statistic <10 and R^2 <0.1%, suggesting that these genotypes may be weak instruments (online supplemental figure S1).

In the Copenhagen City Heart Study, median follow-up was 21 years (up to 38 years) for the observational analyses and 18 years (up to 27 years) for the genetic analyses. In the Copenhagen General Population Study, corresponding values were 8.8 years (up to 14 years) and 9.3 years (up to 15 years), respectively. During

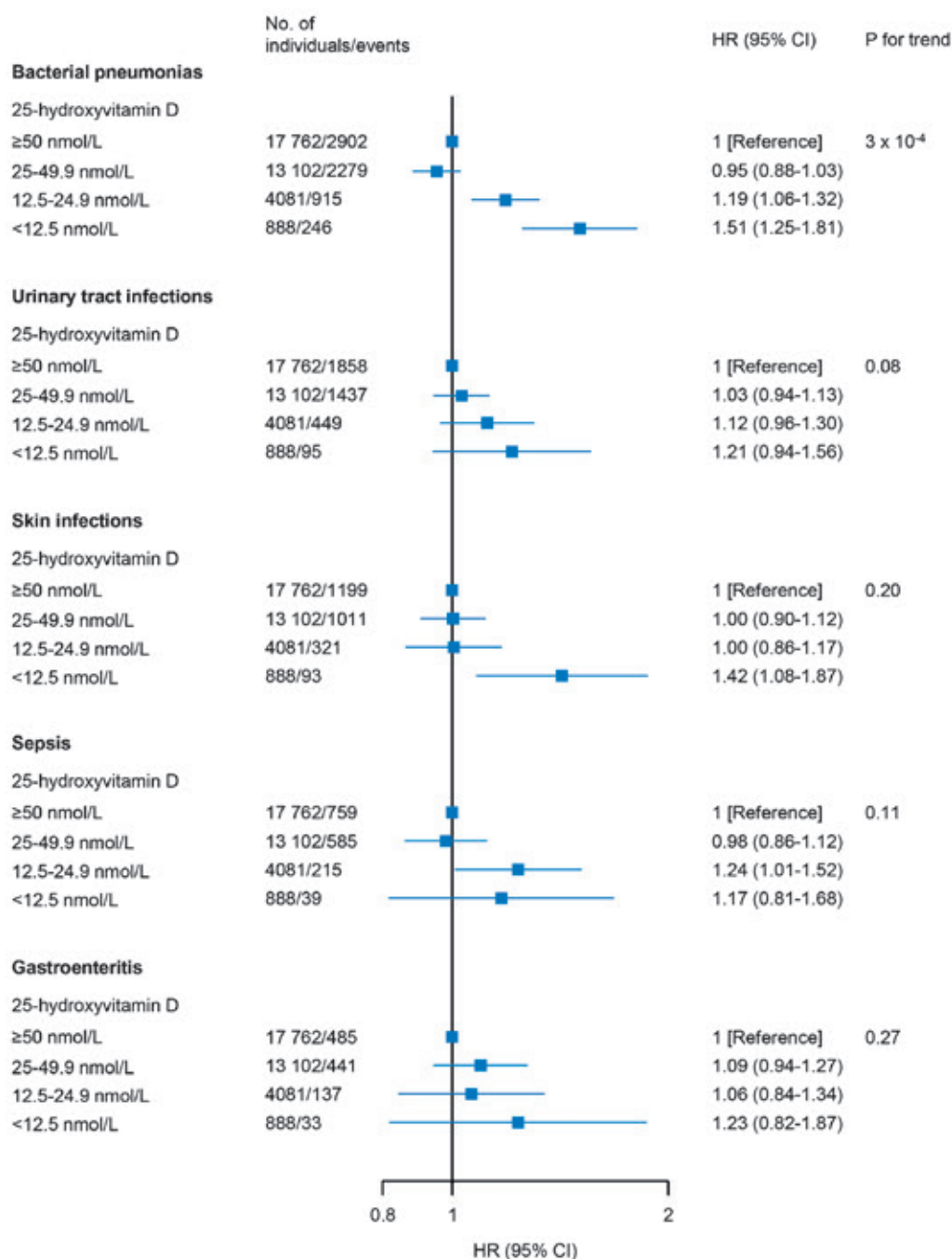


Figure 4 Risk of bacterial pneumonias and other infections according to plasma 25-hydroxyvitamin D concentration in individuals in the general population. Risk estimates obtained from Cox regression models with multiple failure-time analysis according to Andersen and Gill adjusted for age (as timescale), sex, seasonal variation of 25-hydroxyvitamin D, birth year, cohort, body mass index, smoking status, cumulative tobacco consumption, alcohol consumption, estimated glomerular filtration rate, chronic disease at baseline (diabetes, ischaemic heart disease, chronic obstructive pulmonary disease and cancer). Based on the Copenhagen City Heart Study and Copenhagen General Population Study.

follow-up, we observed 6342 bacterial pneumonias in the observational analyses and 13 916 in the genetic analyses.

Observational analyses

Individuals with 25-hydroxyvitamin D < 25 nmol/L had an increased risk of bacterial pneumonias compared with those with 25-hydroxyvitamin D ≥ 25 nmol/L in the Copenhagen City Heart Study and Copenhagen General Population Study, both in combined and separate analyses (figure 2). Compared with individuals with 25-hydroxyvitamin D ≥ 25 nmol/L, the multivariable adjusted HR for bacterial pneumonias was 1.27 (95% CI: 1.16 to 1.40) in individuals with 25-hydroxyvitamin D < 25 nmol/L. This risk estimate was largely similar in subgroup analyses without evidence of interaction, including in the two studies separately (figure 3).

Compared with individuals with 25-hydroxyvitamin D ≥ 50 nmol/L, multivariable adjusted HRs for bacterial pneumonias were

0.95 (95% CI: 0.88 to 1.103) in those with 25 to 49.9 nmol/L, 1.19 (95% CI: 1.06 to 1.32) in those with 12.5 to 24.9 nmol/L and 1.51 (95% CI: 1.25 to 1.81) in those with < 12.5 nmol/L 25-hydroxyvitamin D (figure 4). No clear associations were observed between 25-hydroxyvitamin D concentrations and risk of urinary tract infections, skin infections, sepsis or gastroenteritis (all p values for trend ≥ 0.05); however, 95% CIs for HRs did not overlap with 1.0 in individuals with plasma 25-hydroxyvitamin D < 12.5 nmol/L for skin infections and 12.5–24.9 nmol/L for sepsis.

Genetic analyses

Each 25-hydroxyvitamin D decreasing allele in CYP2R1 rs12794714 and CYP2R1 rs10741657 was associated with higher risk of bacterial pneumonias but not of other infections (figure 5). Other genotypes were not associated individually with higher risk of bacterial pneumonias or other infections; however, individuals

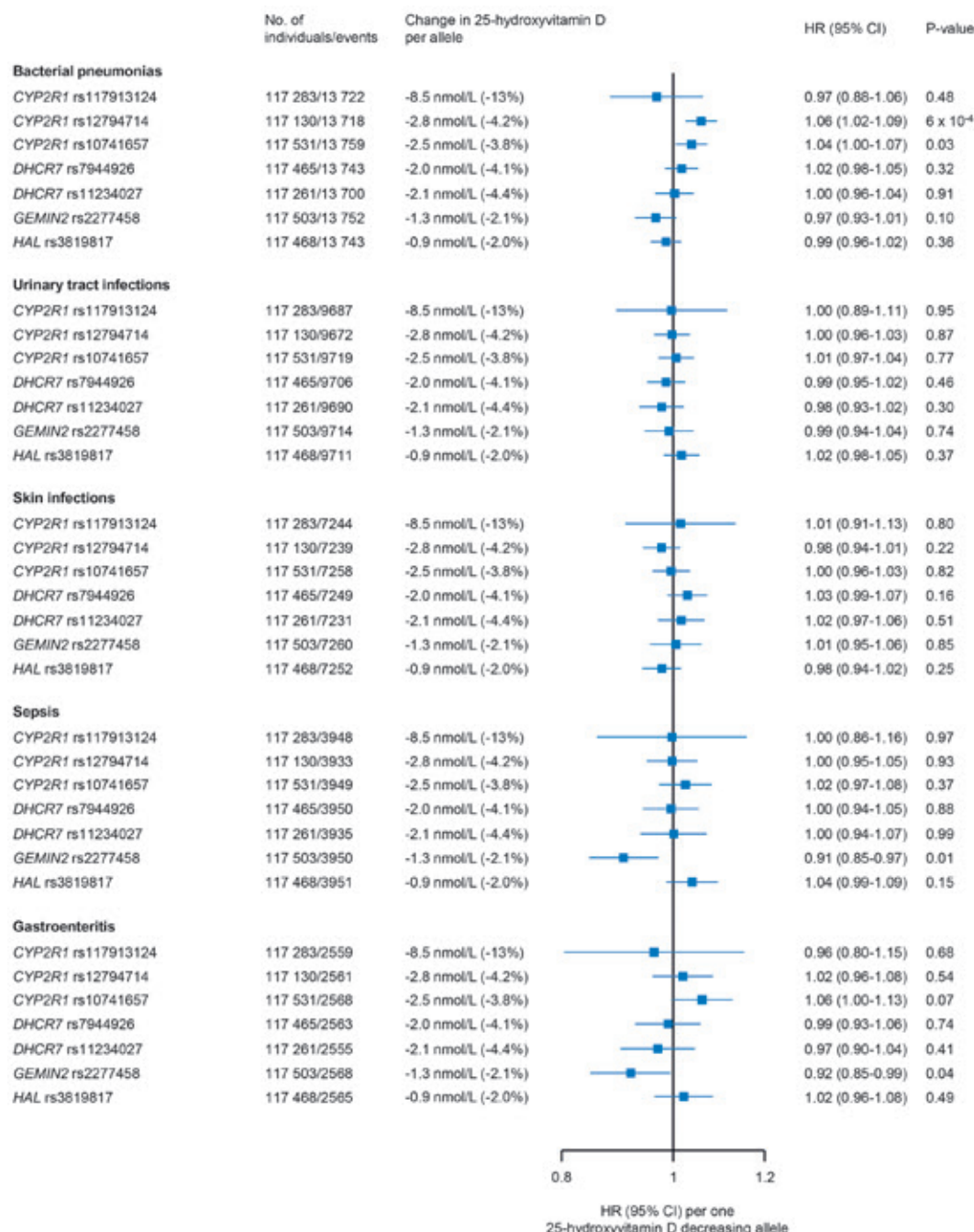


Figure 5 Risk of bacterial pneumonias and other infections according to 25-hydroxyvitamin D decreasing genotypes in individuals in the general population. Risk estimates obtained from Cox regression models with multiple failure-time analysis according to Andersen and Gill adjusted for age (as timescale), sex, birth year and cohort. Based on the Copenhagen City Heart Study and Copenhagen General Population Study.

with genetic variation in GEMIN2 rs2277458 had a reduced risk of sepsis and gastroenteritis.

In instrumental variable analysis, lower plasma 25-hydroxyvitamin D concentration was associated with higher risk of bacterial pneumonia (figures 6 and 7). ORs for bacterial pneumonia per 10nmol/L lower plasma 25-hydroxyvitamin D were respectively 1.12 (95% CI: 1.02 to 1.23) in Wald's ratio, 1.12 (95% CI: 1.04 to 1.20) in inverse-variance weighted, 1.63 (95% CI: 0.96 to 2.78) in MR-Egger and 1.15 (95% CI: 1.05 to 1.26) in weighted median instrumental variable analysis (figure 7, upper panel). Results were attenuated but similar when also including the rare genetic variant, that is, CYP2R1 rs117913124, and the genetic variants prone to weak instrument bias, that is, GEMIN2 rs2277458 and HAL rs3819817 (figure 7, lower panel). However, these latter analyses may be biased as outliers affect inverse-variance weighted and MR-Egger estimates, and weak instruments can also bias estimates in general, though usually towards the observational estimate.

Sensitivity analyses

None of the 25-hydroxyvitamin D decreasing genotypes were associated with risk of influenza and all pneumonia combined in the UK Biobank, and estimates were attenuated for the Copenhagen City Heart Study and Copenhagen General Population Study compared with bacterial pneumonia alone (online supplemental figure S2); however, the CYP2R1 rs12794714 genotype was associated with increased risk of influenza and all pneumonia in the Copenhagen City Heart Study and Copenhagen General Population Study (latter also as a separate cohort). However, this outcomes definition is different from our main analyses as UK Biobank estimates could not be attained for bacterial pneumonia alone.

Since we suspected that the increased risk of bacterial pneumonia was probably driven through CYP2R1, we combined all available genetic variants around CYP2R1 (rs117913124, rs12794714 and rs10741657) in a genetic risk score. HR per 1,25-hydroxyvitamin D decreasing CYP2R1 risk score for bacterial pneumonias was 1.03 (95% CI: 1.01 to 1.04) in an unweighted genetic risk score and

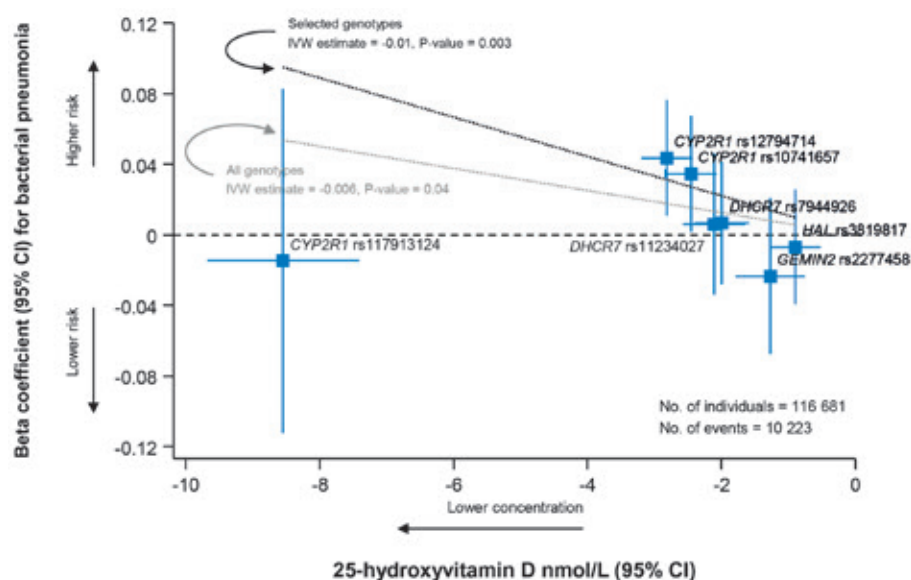


Figure 6 Beta coefficient for bacterial pneumonia and change in plasma 25-hydroxyvitamin D concentration according to 25-hydroxyvitamin D decreasing genotypes in individuals in the general population. Beta coefficients for bacterial pneumonia obtained from logistic regression models adjusted for age, sex, birth year and cohort. Change in plasma 25-hydroxyvitamin D concentration obtained from multiple linear regression models adjusted for age, sex, seasonal variation of 25-hydroxyvitamin D, birth year and cohort. Dashed black (selected genotypes) and grey (all genotypes) lines obtained from inverse-variance weighted (IVW) instrumental variable analysis. Selected genotypes included CYP2R1 rs12794714, CYP2R1 rs10741657, DHCR7 rs7944926 and DHCR7 rs11234027. Based on the Copenhagen City Heart Study and Copenhagen General Population Study.

1.01 (95% CI: 1.00 to 1.02) in a weighted genetic risk score (online supplemental figure S3).

DISCUSSION

By using a Mendelian randomisation approach in two population-based cohorts with >110000 individuals and up to 38 years of follow-up, we found that low plasma vitamin D is associated observationally and genetically with increased risk of bacterial pneumonias. There was no convincing evidence observationally or genetically that vitamin D is associated with risk of urinary tract infections, skin infections, sepsis or gastroenteritis.

Vitamin D seems to regulate the innate immune system, which is essential in the defence against bacterial pneumonias.² It has been shown that toll-like receptor (TLR)-2/1 ligand, T-cell-derived interferon-gamma (IFN- γ) and T-cellexpressed CD40 ligand induce upregulation of vitamin D receptor (VDR) and 25-hydroxyvitamin D-1- α -hydroxylase (CYP27B1) (enzyme that converts inactive 25-hydroxyvitamin D to active 1,25-hydroxyvi-

tamin D) through an autocrine pathway in human monocytes and macrophages.^{39–43} VDR and 1,25-hydroxyvitamin D then induce upregulation of cathelicidin-related antimicrobial peptides, that is, LL-37 and hCAP-18, and human β -defensin 2, autophagy and phagosome maturation, which ultimately leads to intracellular killing of pathogens, as shown for *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Bordetella bronchiseptica*.^{39–44}

Interestingly, genetic variants around CYP2R1 but not around DHCR7, GEMIN2 or HAL were associated individually with increased risk of bacterial pneumonias. A potential explanation may be that CYP2R1 is more directly involved in the formation of 1,25-hydroxyvitamin D compared with the other genes. Also, while the role of DHCR7 in vitamin D metabolism is well-established (conversion of 7-dehydrocholesterol to cholesterol), the role of GEMIN2 and HAL is unknown. This may also explain the low F-statistic and R^2 of genetic variants around GEMIN2 and HAL compared with genetic variants around CYP2R1 and DHCR7. Also, we cannot exclude that variants in these genes may only indirectly affect 25-hydroxy-

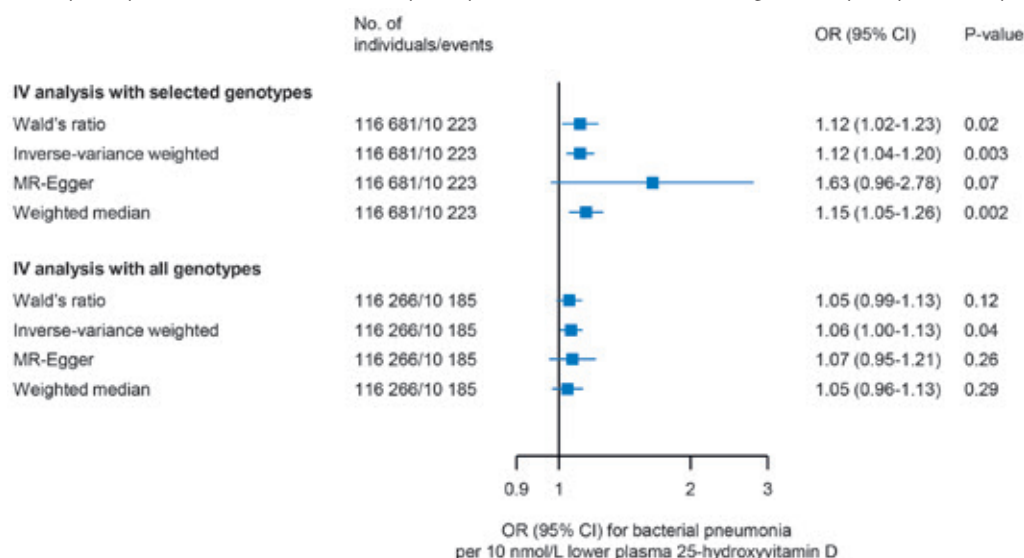


Figure 7 Risk of bacterial pneumonia according to 25-hydroxyvitamin D concentration using instrumental variable analysis in individuals in the general population. Risk estimates for bacterial pneumonia obtained from logistic regression models adjusted for age, sex, birth year and cohort. Change in plasma 25-hydroxyvitamin D concentration obtained from multiple linear regression models adjusted for age, sex, seasonal variation of 25-hydroxyvitamin D, birth year and cohort. Selected genotypes included CYP2R1 rs12794714, CYP2R1 rs10741657, DHCR7 rs7944926 and DHCR7 rs11234027. Based on the Copenhagen City Heart Study and Copenhagen General Population Study. IV, instrumental variable.

vitamin D through pathways affecting both exposure an outcome separately (pleiotropy). Further, we cannot exclude the possibility that vitamin D may not be involved in the defence against bacterial pneumonias, and that other mechanisms involving CYP2R1 may be at play. Finally, the association of genetic variants around CYP2R1 with risk of bacterial pneumonias could represent a chance finding and therefore needs independent confirmation.

We were unable to replicate our findings in the UK Biobank. A potential explanation may be that a different outcome was used, that is, influenza and all pneumonia combined instead of bacterial pneumonia alone. Although we found evidence of an association with influenza and all pneumonia for the 25-hydroxyvitamin D decreasing CYP2R1 rs12794714 genotype, we believe that this association was probably driven by bacterial pneumonia. Another potential explanation may be that events were identified differently in the UK Biobank and the validity of these endpoints in UK biobank is unknown. Information on events in the present study was obtained from the national Danish Patient Registry, which records all public and private hospital contacts in Denmark, where diagnoses are made by medical doctors according to national law. In addition, events were only defined as acute emergency department visits and hospital admissions based on the primary diagnosis to increase the validity,⁴⁵ and to secure that we only captured the most serious cases admitted to hospital in the Copenhagen cohorts.

Previous observational studies have found an association of low plasma 25-hydroxyvitamin D concentrations with higher risk of respiratory tract infections.^{3–9} Although results from randomised controlled trials testing the effect of vitamin D supplementation against respiratory tract infections have been conflicting,^{10–23} the largest systematic review and meta-analysis to date has pointed at a beneficial effect.²⁴ In the present study, we show that low vitamin D is associated with increased risk of bacterial pneumonias in observational analyses and in part in genetic analyses. The fact that there was no evidence observationally or genetically that vitamin D is associated with risk of other organ specific infections suggests that the association could be specific for respiratory infections such as bacterial pneumonias. Future randomised controlled trials should therefore be designed to study the effect of vitamin D supplementation against bacterial pneumonias.

Strengths of the present study include two large-scale independent cohorts from the general population with randomly selected individuals with a long follow-up time, no losses to follow-up and a substantial number of bacterial pneumonias. Furthermore, we used physician encoded diagnoses with high validity.⁴⁶

Potential limitations in Mendelian randomisation analyses include population stratification bias, genetic pleiotropy and linkage disequilibrium. However, as we had an ethnically homogeneous white Danish population, the complicating effects of population stratification were likely avoided, and as genotype distributions did not appear to differ according to the HardyWeinberg equilibrium, genotyping and population sampling errors were also likely avoided.

Another potential limitation is that we did not have information on family relationships, which could bias our results. However, since we have sampled randomly, we believe that this bias will be of minor importance.

As mentioned above, we cannot exclude that our positive genetic observation could not represent a chance finding. However, it is reassuring that all different instrumental variable analyses that were used to estimate the causal effect, each with its belonging strengths and weaknesses,³⁴ gave similar results, suggesting that the results are robust.

In conclusion, low vitamin D is associated observationally and genetically with increased risk of bacterial pneumonias.

Collaborators NA.

Contributors YÇ and SA had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses. YÇ, BGN and SA contributed to the study concept and design. YÇ, BGN and SA collected, analysed or interpreted the data. YÇ wrote the draft manuscript and did the statistical analyses. YÇ, BGN and SA revised the manuscript for important intellectual content. BGN obtained funding. BGN provided administrative, technical or material support. BGN and SA supervised the study.

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Competing interests YÇ reports personal fees from Boehringer Ingelheim, AstraZeneca, Sanofi Genzyme outside the submitted work. BGN and SA have nothing to disclose.

Patient consent for publication Not required.

Ethics approval Both studies were approved by Danish ethical committees and conducted according to the Declaration of Helsinki (approval numbers: KF-V-100- 2039/91 and H-KF-01-144/01). All individuals provided written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Additional data regarding technical details, statistical code and derivative data are available from the corresponding author. Data access for further analyses is possible through direct collaborative agreement or through locally managed access arranged through the study's principal investigator.

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Avaliar o impacto da vacina pneumocócica conjugada 13-valente sobre o empiema infantil na Austrália

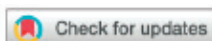
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RESUMO

Contexto O empiema é uma complicação grave de pneumonia causada frequentemente por *Streptococcus pneumoniae* (SP). Avaliámos o impacto da vacina pneumocócica conjugada 13-valente (VPC13) na pneumonia e empiema infantis após a sua inclusão no Programa Nacional de Vacinação Australiano.

Métodos Para hospitalizações por pneumonia bacteriana e empiema, determinámos as taxas de incidência (TI) utilizando a Base de Dados Nacional de Morbilidade Hospitalar, os códigos de alta hospitalar da Classificação Estatística Internacional de doenças e os denominadores populacionais relevantes e calculámos as taxas de incidência (TI) comparando o período de VPC13 (junho 2012 - maio 2017) com o período de VPC7 (junho 2007 - maio 2011). As culturas de sangue e fluido pleural (FP) e o PCR de FP de 401 crianças com empiema de 11 hospitais australianos durante o período de VPC13 foram comparadas com o nosso estudo anterior no período de VPC7.

Achados Durante os períodos de VPC7 e VPC13, as TI por milhão de crianças (95% CI) foram 1605 (1588 a 1621) e 1272 (1259 a 1285) para hospitalizações por pneumonia bacteriana e 14,23 (12,67 a 15,79) e 17,89 (16,37 a 19,42) para hospitalizações por empiema. Os RTI foram 0,79 (0,78 a 0,80) para pneumonia bacteriana e 1,25 (1,09 a 1,44) para empiema. Dos 161 casos de empiema com serotipos SP, 147 (91,3%) foram tipos de vacinas. O ST3 representou 76,4% dos serotipos identificados no período de VPC13, mais do dobro do período de VPC7 ($p < 0,001$); O ST19A diminuiu de 36,4% para 12,4%. Não foram identificados casos de empiema ST1 no período VPC13 contra 14,5% no período VPC7.

Interpretação A VPC13 resultou numa redução significativa das hospitalizações por todas as causas de pneumonia bacteriana, mas as hospitalizações por empiema aumentaram significativamente, com o aparecimento do pneumococo ST3 como o serotipo dominante no empiema.

Número de registo do ensaio Registo de Ensaios Clínicos da Austrália e Nova Zelândia ACTRN 12614000354684.

INTRODUCTION

Empyema, the collection of pus in the pleural cavity, is a complication which affects approximately 1% of children hospitalised with pneumonia.¹⁻³ The most common bacterial cause of empyema is *Streptococcus pneumoniae* (SP); other bacterial causes include methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pyogenes* and *Haemophilus influenza*.¹

In January 2005, the 7-valent pneumococcal conjugate vaccine (7vPCV) was introduced onto the National Immunisation Program (NIP) for all Australian children. 7vPCV provides coverage against invasive pneumococcal disease (IPD) caused by serotypes (ST) 4, 6B, 9V, 14, 18C, 19F and 23F, which were responsible for up to 70% of paediatric IPD at the time of introduction.⁴ We previously reported a decline in pneumonia hospitalisations in children after the 7vPCV was

Key messages

What is the key question?

► What was impact following the introduction of the 13-valent pneumococcal conjugate vaccine (13vPCV) on childhood pneumonia and empyema in Australia?

What is the bottom line?

► 13vPCV resulted in a decrease in childhood pneumonia hospitalisations with a contemporaneous increase in admissions for empyema. Although there was a substantial reduction in serotype (ST) 1, ST 3 is now the predominant causative organism of childhood empyema with the suggestion of emergence of non-vaccine STs.

Why read on?

► Childhood bacterial pneumonia and empyema are potentially preventable diseases through vaccination. Our study highlights the potential negative impact of 13vPCV on childhood empyema by potentially causing ST replacement disease, emphasising the need for enhanced molecular surveillance following the introduction of new vaccines onto national programmes.

introduced in Australia, with a contemporaneous increase in empyema, especially in children aged 1–4 years old.² Increases in empyema following the introduction of 7vPCV were also reported in other countries.⁵⁻⁷ The reason for the increase is uncertain, but it has been speculated that immunisation with 7vPCV resulted in the selective carriage in the nasopharynx of non-vaccine pneumococcal ST with a predilection for invasion of the pleural space, particularly ST1, 3 and 19A.^{1 5-7}

In July 2011, the 13-valent pneumococcal conjugate vaccine (13vPCV) replaced the 7vPCV on the Australian NIP, extending coverage to six additional ST 1, 3, 5, 6A, 7F and 19A. Doses of 13vPCV were scheduled at 2, 4 and 6 months, with a booster dose at age 12–18 months only recommended for medically high risk and Indigenous children. At the time of 13vPCV introduction, a time-limited booster dose of 13vPCV was available for children aged 12–35 months of age who had already completed the primary course of 7vPCV.

Most studies of the impact of 13vPCV in Europe and North America have reported declines in IPD, including bacterial pneumonia and empyema.⁸⁻¹¹ International evidence also highlights reductions in the prevalence of IPD caused by 13vPCV ST.^{8 9 12-15}

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Table 1 Paediatric bacterial pneumonia and empyema coded hospitalisation in Australia in the 7-valent pneumococcal conjugate vaccine and 13-valent pneumococcal conjugate vaccine periods

Age group (years), by disease category	June 2012–May 2017 hospitalisations (no.)	Incidence/1 000 000 children per year (95%CI)	June 2007–May 2011 hospitalisation (no.)		Incidence/1 000 000 children per year (95%CI)	Incidence rate ratio (95%CI)
			Expected	Observed		
Bacterial pneumonia						
0–19	36 087	1605 (1588 to 1621)	46 925	37 587	1272 (1259 to 1285)	0.79 (0.78 to 0.80)
<1	3622	3128 (3025 to 3229)	4801	3091	1998 (1927 to 2068)	0.64 (0.60 to 0.67)
1–4	17 595	3968 (3909 to 4026)	24 029	17 641	2870 (2828 to 2913)	0.72 (0.70 to 0.74)
5–9	7780	1437 (1406 to 1470)	10 500	9111	1218 (1193 to 1243)	0.84 (0.82 to 0.87)
10–14	3493	624 (604 to 645)	4370	3773	537 (519 to 554)	0.85 (0.82 to 0.9)
15–19	3597	611 (591 to 631)	4484	3971	540 (523 to 557)	0.88 (0.84 to 0.92)
Empyema						
0–19	320	14.23 (12.67 to 15.79)	409	529	17.89 (16.37 to 19.42)	1.25 (1.09 to 1.44)
<1	49	42.31 (30.46 to 54.15)	64	50	32.31 (23.35 to 41.27)	0.73 (0.51 to 1.13)
1–4	157	35.40 (29.86 to 40.94)	212	255	41.49 (36.39 to 46.58)	1.17 (0.96 to 1.42)
5–9	36	6.65 (4.47 to 8.82)	51	96	12.83 (10.27 to 15.40)	1.92 (1.31 to 2.83)
10–14	27	4.82 (3.00 to 6.64)	35	55	7.82 (5.75 to 9.89)	1.62 (1.02 to 2.56)
15–19	51	8.86 (6.28 to 11.04)	66	73	9.92 (7.65 to 12.20)	1.14 (0.80 to 1.63)

We aimed to investigate the impact of the introduction of 13vPCV on childhood empyema in Australia by (1) examining the rates of pneumonia and empyema hospitalisations in children using national hospitalisation data, and (2) by prospectively identifying the bacterial causes of empyema, in particular strains of SP, through targeted enhanced molecular surveillance.

METHODS

Empyema

We analysed hospitalisations for childhood empyema using data from a whole-of-population national administrative dataset (National Hospital Morbidity Database) compiled by the Australian Institute of Health and Welfare (<https://www.aihw.gov.au/>). This included data on hospitalisations for all Australian children aged 0–≤19 years from 1 January 2007 to 30 June 2017. The data cubes available were grouped <1 years, 1–4 years, 5–9 years, 10–14 years and 15–19 years. International Statistical Classification of Disease and Related Health Problems (ICD-10-AM) diagnosis codes were used to identify all hospitalisations with principal diagnosis codes associated with empyema (J86.0 and J86.9). Age-specific mid-year population estimates were sourced from Australian Bureau of Statistics 2016 census estimates.^{16,17} Incidence rates were calculated for the 13vPCV period (1 June 2012–31 May 2017) and compared with those for the 7vPCV period (1 June 2007–31 May 2011) as per our previous evaluation.²

From February 2015 to September 2018, we prospectively identified and enrolled children (0–17 years old) hospitalised to 1 of 11 sentinel paediatric hospitals across Australia with empyema. This was defined as having one or more of the following clinical symptoms: cough, fever or increased work of breathing; in addition to endpoint consolidation on chest X-ray as judged by the treating physician; presumed infective aetiology requiring antibiotic treatment (either planned or commenced) and presence of pus on microscopy on drained

pleural fluid or a culture positive pleural fluid result. Children were excluded if their primary reason for admission was not respiratory illness. Consent was obtained from the primary caregiver. We have previously undertaken a similar prospective study using the same methodology in the 7vPCV period era where we recruited children with empyema over a 2-year period (2007–2009).¹

Demographic and clinical data were obtained from review of the medical record including age, sex, Aboriginal and/or Torres Strait Islander status, area of residence and risk factors for IPD. The vaccination status of enrolled patients was obtained by either review of the child's hand-held records or the Australian Immunisation Registry. Children were considered either fully, partially or not vaccinated with pneumococcal conjugate vaccine at the time of hospitalisation according to the NIP vaccination schedule.

The microbiological methods have been described previously.¹ Briefly, we collected blood and pleural fluid specimens from each enrolled child. Blood and pleural fluid specimens were cultured at local hospitals by standard local laboratory methods. A separate aliquot (ideally 10 mLs) of pleural fluid was stored at –20°C. Specimens were then transported in batches on dry ice by a commercial transport company to the Centre for Infectious Disease and Microbiology Laboratory, Westmead Hospital, New South Wales, for processing.

DNA was extracted from pleural fluid specimens using NucliSENS easyMAG Total Nucleic Acid Extractor (bioMérieux Australia Pty, Sydney, New South Wales, Australia). DNA specimens were tested with real-time quantitative PCR using Rotor-Gene 6000 real time DNA amplification system (Qiagen, Australia) targeting autolysin gene (*lytA*) for pneumococcal DNA detection as described by McAvine *et al.*¹⁸ Standard curves and minimal limit of detection were generated by plotting the cycle threshold values of the qPCR performed on a dilution series of synthetically synthesised *lytA* (*lytA* gBlock, IDT Technologies, Australia) in triplicate. All assays with each clinical sample were performed in duplicate. A specimen was considered positive if one of the two duplicates gave a positive result within the <40-cycle cut-off.

All samples in which pneumococcus was detected by PCR were further tested to determine the specific pneumococcal ST. Sequencing PCR was used to amplify and sequence *cpsB* capsular gene.¹⁹ Sequencing results were confirmed with ST-specific PCR^{20,21} based on ST shown in sequencing.

Pleural fluid samples were also tested for MSSA, MRSA and *H. influenzae* by PCR using the BD MAX StaphSR assay (BD Diagnostics, Québec, Canada) which targets SA (SCCmec) and MRSA (*mecA* and *mecC*)-specific genome.²² *H. influenzae* was detected using conventional PCR methods with primers: Hinf OmP 6F, 5'-AAT GGT GCT GCT CAA ACT TT-3'; and Hinf OmP 6R, 5'-TCT AAG ATT TGA ACG TAT TCA CC-3' as previously published.¹

Annual incidence rates (IR) were calculated for each of the age groups (<1, 1–4, 5–9, 10–14 and 15–19 years old) by dividing the year-specific total number of hospitalisations (bacterial pneumonia and empyema) in that specific age group by the total midyear age-specific

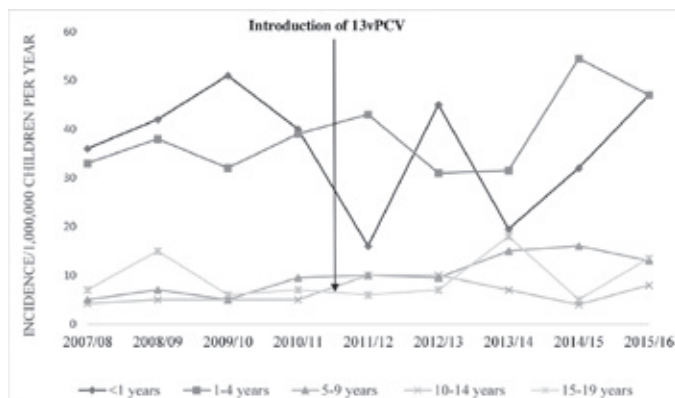


Figure 1 Year-specific and age-specific incidence rates of paediatric empyema hospitalisation in the 7-valent pneumococcal conjugate vaccine (2007–2011) and 13-valent pneumococcal conjugate vaccine (13vPCV; 2012–2016) periods in Australia.

Table 2 Characteristics of children with empyema in Australia in the 7-valent pneumococcal conjugate vaccine (7vPCV; 2007–2009) and 13-valent pneumococcal conjugate vaccine (13vPCV) (2015–2018) enhanced surveillance periods

	Participants	
	April 2007–April 2009 (7vPCV period)	February 2015–September 2018 (13vPCV period)
Number	172	401
Age (years; median, range)	3.9 (0.4–15.5)	4.0 (0.3–17.3)
Sex		
Male	93 (54.1)	208 (51.9)
Indigenous status		
Indigenous	8 (4.7)	31 (7.7)
Non Indigenous	160 (93)	366 (91.3)
Not recorded	4 (2.3)	4 (1)
State/territory		
Queensland	59 (34.3)	50 (12.5)
New South Wales	52 (30.2)	127 (31.7)
Victoria	37 (21.5)	132 (32.9)
Western Australia	16 (9.3)	33 (8.2)
South Australia	4 (2.3)	39 (9.7)
Tasmania	2 (1.2)	5 (1.2)
Australian Capital Territory	2 (1.2)	9 (2.2)
Northern Territory	0	6 (1.5)
Chronic respiratory diseases	7	14
Congenital diseases	3	17
Potentially immuno-compromised	6	12
Cardiac disease	1	1

*Values are no. (% of total cases) unless otherwise indicated.

population. We calculated an expected number of hospitalisations in the 13vPCV period by applying the estimated rate in the 7vPCV period (1 June 2007–31 May 2011) to the mid-year population in the 13vPCV period (1 June 2012–31 May 2017). Poisson estimation was used to estimate incident rate ratio (IRR) and corresponding 95% CIs in the 13vPCV period compared with the 7vPCV period. We described the frequency of pathogen and ST and compared this to the frequency previously described by us for the 7vPCV era using a χ^2 test.¹

Pneumonia

We used the same methods described above to analyse hospitalisations for childhood pneumonia. ICD-10-AM diagnosis codes were used to identify all hospitalisations with principal diagnosis codes associated with bacterial pneumonia (J13–J15.9, J17.0, J18.0, J18.1, J18.8 and J18.9). As describe above, incidence rates were calculated for the 13vPCV period (1 June 2012–31 May 2017) and compared with those for the 7vPCV period (1 June 2007–31 May 2011) as per our previous evaluation.²

RESULTS

National hospitalisations

The mean annual incidence rates/1 000 000 children per year (95%CI) in the 7vPCV and 13vPCV periods were 1605 (1588 to 1621) and 1272 (1259 to 1285) for hospitalisations coded as bacterial pneumonia, and 14.23 (12.67 to 15.79) and 17.89 (16.37 to 19.42) for hospitalisations coded as empyema, respectively (table 1).

The mean annual IR of hospitalisations coded as bacterial pneumonia reduced by 21% (IRR 0.79, 95%CI 0.78 to 0.80) and those coded as empyema increased by 25% (IRR 1.25, 95%CI 1.09 to 1.44) in the 13vPCV in comparison to the 7vPCV period (table 1 and figure 1). The age-specific number of hospitalisations and incidence rates with rate ratios are presented in table 1. There was a decline in the observed, compared with expected, number of admissions with bacterial pneumonia-coded hospitalisations in the 13vPCV period across all age groups, with the greatest decline in children <5 years old (table 1). However, the observed, compared with expected, number of empyema coded hospitalisations increased in the 13vPCV period in all age-groups except in children <1year old (table 1).

Targeted enhanced surveillance of empyema

A total of 401 children with empyema were enrolled; of these, 208 (52%) were male and the median age was 4.0 (range 0.3–17.3) years (table 2).

Of the 401 with empyema, 300 (75%) had both pleural fluid and blood cultured, 91 (23%) only had pleural fluid cultured, 7 (1.7%) only had blood cultured and 3 (0.7%) participants had neither cultured. Of 307 blood cultures, 35 (11%) were positive for one or more bacterial pathogens; of 391 pleural fluid cultures, 118 (30%) were positive (table 3).

SP was the most common causative organism identified in both blood and pleural fluid. SP was identified in the pleural fluid in 55%

Table 3 Prevalence and value of dissaving in the years prior to and after TB-treatment completion, stratified by wealth quintiles*

	April 2007–April 2009 (7vPCV period)			February 2015–Sept 2018 (13vPCV period)		
	Blood Culture	Pleural Fluid Culture	PCR	Blood Culture	Pleural Fluid Culture	PCR
Organism	(Total number =152) %, (n=)	(Total number=160) %, (n=)	(Total number=145) %, (n=)	(Total number=307) %, (n=)	(Total number=391) %, (n=)	(Total number=337) %, (n=)
<i>Streptococcus pneumoniae</i>	12.5 (19)	7.5 (12)	51 (74)	4.6 (14)	9.7 (38)	54.9 (207)
<i>Streptococcus pyogenes</i>	2.0 (3)	8.8 (14)	–	2.9 (9)	9.5 (37)	–
<i>Streptococcus milleri</i>	–	2.5 (4)	–	–	1.3 (5)	–
Methicillin susceptible <i>Staphylococcus aureus</i>	0.7 (1)	6.8 (11)	4.1 (6)	1 (3)	4.1 (16)	6.6 (25)
Methicillin-resistant <i>Staphylococcus aureus</i>	0.7 (1)	3.8 (6)	4.8 (7)	0.7 (2)	2.8 (11)	0.3 (1)
Coagulase negative staphylococci	2.6 (4)	1.3 (2)	–	0.7 (2)	1.3 (5)	–
<i>Haemophilus influenzae</i>	0.7 (1)	–	2.8 (4)	0.3 (1)	0.8 (3)	1.8 (7)
<i>Mycobacterium tuberculosis</i>	–	0.6 (1)	–	–	–	–
<i>Pseudomonas aeruginosa</i>	–	0.6 (1)	–	–	–	–
<i>Mycoplasma pneumoniae</i>	–	–	0.7 (1)	–	–	–
<i>Chlamydia pneumoniae</i>	–	–	0.7 (1)	–	–	–
<i>Viridans streptococci</i>	–	–	–	–	0.8 (3)	–
<i>Escherichia Coli</i>	–	–	–	–	0.5 (2)	–
Others*	2.6 (4)	2.5 (4)	–	1.3 (4)	1 (4)	–

7vPCV period.

Blood cultures: one isolate each of *Streptococcus sanguinis*; *Staphylococcus hominis* (from a specimen in which *S. aureus* was also isolated); *Neisseria meningitidis*; and

Actinomyces naeslundii.

Pleural fluid: one isolate each of *Streptococcus oralis*; *Staphylococcus cohnii*; *Eikenella corrodens*; and *Bacteroides fragilis* (the last two from the same specimen, from which *S. milleri* was also isolated). One pleural fluid specimen was culture positive for both MRSA and MSSA.

13vPCV period.

Blood cultures: one isolate each of Gram-positive *Bacilli*; *Bacillus endoradicus*; Gram-positive staphylococci; and *Micrococcus luteus*.

Pleural fluid: one isolate each of *Brevundimonas diminuta*; *Kocuria kristinae*; *A. odontolyticus* and *Fusobacterium necrophorum*. Both MSSA and MRSA were cultured from one pleural fluid specimen; both *Streptococcus pyogenes* and *H. influenzae* in one specimen; both *S. pneumoniae* and *Escherichia coli* in one pleural fluid sample; both *E. coli* and *S. milleri* in one pleural fluid sample and; one pleural fluid sample had MSSA, *A. odontolyticus* and *V. streptococci* isolated.

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

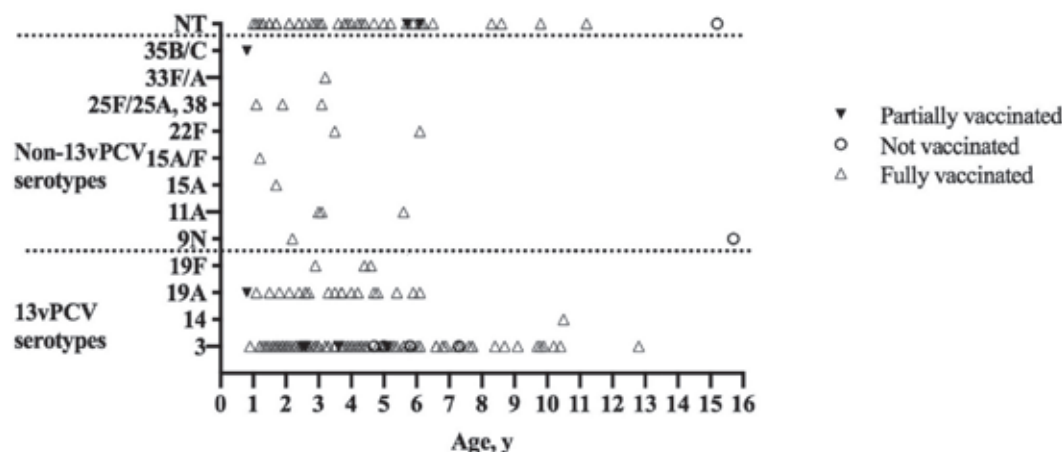


Figure 2 *Streptococcus pneumoniae* serotype distribution in relation to age and vaccination status of children with empyema Australia, in the 13-valent pneumococcal conjugate vaccine (13vPCV) period, 2015–2018. NT, not able to be typed.

by PCR and in 10% by culture and was detected in 5% by blood culture. Other organisms detected included *S. pyogenes*, MSSA, and MRSA (table 3).

Of the 207 children with SP-confirmed empyema, 191 (92%) were fully vaccinated against *pneumococcus* (mean age 4.0 years; range 0.9–12.8 years), 10 (4.8%) were partially vaccinated (mean age 3.3 years; range 0.8–6.1 years), and 6 (2.9%) were unvaccinated (mean age 9.0 years; range 4.7–15.7 years; figure 2).

Of 207 SP PCR positive samples, ST were identified in 161 (78%) (table 4). Forty-six (22%) samples were not able to be typed. 13vPCV ST3 accounted for 76% of all identified ST in the 13vPCV period, compared with 33% in the 7vPCV period ($p < 0.001$). ST19A accounted for 12% of identified ST, compared with 36% in the 7vPCV era. No cases of ST1 were identified, compared with 15% in the 7vPCV period. A small number of non-13vPCV ST were identified which were not identified in the 7vPCV era (table 4).

Table 4 *Streptococcus pneumoniae* serotypes identified in PCR positive specimens from children with empyema, Australia, in the 7-valent pneumococcal conjugate vaccine (7vPCV) (2007–2009) and 13-valent pneumococcal conjugate vaccine (13vPCV) periods

Serotypes	No. (%) specimens	
	April 2007–April 2009 (7vPCV period)	February 2015–Sept 2018 (13vPCV period)
Total number serotypes	55	161
13vPCV serotypes	50%, (n=)	147 %, (n=)
3	32.7 (18)	76.4 (123)
19A	36.4 (20)	12.4 (20)
19F	–	1.9 (3)
14	1.8 (1)	0.6 (1)
1	14.5 (8)	–
7F/7A	3.6 (2)	–
9V/9A	1.8 (1)	–
Non-vaccine serotypes	5 %, (n=)	14 %, (n=)
11A	–	1.9 (3)
25F/25A, 38	–	1.9 (3)
9N	–	1.2 (2)
22F	–	1.2 (2)
15A	–	0.6 (1)
15A/F	–	0.6 (1)
33F/A	–	0.6 (1)
35B/35C	–	0.6 (1)
22F/22A	3.6 (2)	–
6C	1.8 (1)	–
15F	1.8 (1)	–
21	1.8 (1)	–

*7vPCV period: two serotypes were identified in each of three specimens: 19A/3, 19A/1 and 6C/15F. The multiplex PCR reverse line blot assay cannot distinguish some pairs or groups of closely related serotypes, including 7F/7A, 22F/22A, serogroup 6. Individual serotypes within serogroup six were distinguished by using serotype-specific PCR.

*13vPCV period: serotypes 25F, 25A and 38 were grouped together as they could not be individually identified through molecular methods.

DISCUSSION

After the introduction of 13vPCV in Australia, there was a 25% increase in hospitalisations in children coded as empyema and a 21% decrease in hospitalisations coded as bacterial pneumonia. Our incidence data for childhood empyema hospitalisations are similar to that reported in other countries.^{10,23,24} Contemporaneous data from our enhanced surveillance study at sentinel hospitals indicate that pneumococcus remains the most common cause of empyema in Australian children in the 13vPCV period, followed by *Streptococcus pyogenes*, MSSA and MRSA. The 13vPCV ST ST3 now accounts for 76% of pneumococcal empyema in children, compared with 33% in the 7vPCV period. Despite a reduction in the prevalence of ST19A, it remains the second most common identified ST. The 13vPCV ST ST1 appears to have been eliminated as an important cause of empyema among Australian children. Only 9% of pneumococcal ST identified were non-13vPCV ST.

The observed reduction in incidence of bacterial pneumonia coded hospitalisations is consistent with other data from Australia. Jayasinghe *et al* previously reported declines in the rates of overall IPD in the 13vPCV period in Australia, which were greatest in children aged less than 2 years old.²⁵ Our study contrasts with data reported from some other countries; for example, a decrease in both pneumococcal pneumonia and empyema was observed after the introduction of the 13vPCV in the USA.^{8,10} In France, the frequency of pneumococcal pleural effusion and empyema declined from 79% to 36%.¹¹ Similarly, Spain⁹ and Scotland²⁶ also observed declines in empyema rates; Sweden reported a significant decline in pneumonia hospitalisations of 19%²³ and Argentina reported a 51% decrease in community acquired pneumonia, 39% decrease in empyema and 68% decrease in pneumococcal empyema after the 13vPCV introduction.²⁴

We acknowledge that a potential limitation of our empyema data is relatively small numbers which may be subject to natural variations in discrete clusters of ST-specific disease.

In Europe and the USA, a range of changes in specific pneumococcal ST causing pneumonia and empyema have been reported following the introduction of 13vPCV. Greece¹⁵ and Germany¹³ have reported a predominance of empyema caused by ST3. Sweden reported a decline in ST6A and ST19A but no change in ST3.¹² Shortly after the introduction of the 13vPCV in Spain, the most common ST causing pneumococcal pneumonia and empyema was ST1 accounting for 42% of cases.⁹ A Portuguese study¹⁴ also reported a predominance of ST1, in addition to ST3 and 19A in empyema post 13vPCV, suggesting reduced vaccine effectiveness. In the USA, early reports using a 4+0 schedule suggested a fall in invasive pneumococcal infection caused by ST3 and 19A;²⁷ however, more recent studies suggest that they were reportedly still responsible for half of pneumococcal pneumonia and empyema cases post 13vPCV.⁸ It is worth noting that the limitations of making these international comparisons are the different vaccination schedules as well the time period of analysis following introduction of 13vPCV.

There are several plausible explanations for the observed increase in empyema hospitalisations in our study. While changes in coding practices could account for the increase, targeted molecular

surveillance suggests that the rise has been driven by the increased prevalence of ST ST3 which has previously been reported to have a predilection for infection of the pleural space and causes complicated pneumonia worldwide.^{13,14} Although included in 13vPCV, there have been reports of suboptimal protection¹³ and vaccine failures.²⁸ We note previous reports of the apparent suboptimal vaccine protection of 13vPCV against ST ST3^{14,29} which has been shown to produce and release more capsular polysaccharide than other 13vPCV pneumococcal ST. The capsular polysaccharide is thought to interfere with antibody-mediated killing, reducing the protective efficacy of pre-existing anticapsular antibody,³⁰ suggesting that higher antibody titres are required for protection than for other strains.³¹ This may be particularly relevant in empyema fluid where the bacterial load and thus the amount of capsular polysaccharide is likely to be high.

Furthermore, Zimmerman *et al* reported that children vaccinated with an unboosted '3+0' schedule at 2, 4 and 6 months of age were found to have waning protective pneumococcal antibody concentrations at 13 months of age.²⁹ We speculate that waning from the unboosted 3+0 NIP schedule in place in Australia during the period of our study may have contributed to the high prevalence of 13vPCV pneumococcal ST causing empyema cases in our study. The Australian NIP has since moved to a boosted '2+1' (2, 4 and 12 months) schedule in response to persisting rates of 13vPCV ST IPD.³² However, it is possible that this will have minimal impact on ST3, as demonstrated in countries that have used a 2+1 schedule since inception.^{12–15} It is unlikely that the increase in our study is due to lack of vaccination coverage, given only 2.9% of cases in the molecular surveillance arm of the study were unvaccinated.

Our study has several limitations, including the inherent limitation of hospital discharge codes for ascertaining disease rates. First, the inclusion of 'principal' diagnosis discharge codes only, and not secondary codes means that we may have underestimated the true rates. Second, we were unable to link all hospitalised cases to microbiology data to validate whether hospitalisations coded as bacterial pneumonia or empyema were truly associated with confirmed bacterial infection; it is known that laboratory test results are not necessarily reflected in hospital coding. However, unlike uncomplicated pneumonia which may be caused by either viruses or bacteria, empyema is thought to be exclusively caused by bacterial infection. Not all childhood empyema cases in Australia were captured by targeted surveillance over the study period, as many children were likely treated outside participating hospitals; we enrolled children from all Australian states and territories to try to obtain a nationally representative sample.

Our results are also in keeping with a recent small Australian study which reported an increase in ST3 empyema in the 13vPCV era in one centre.³³ In further support of our findings, Jayasinghe *et al* studied the impact of 13vPCV on IPD compared with the 7vPCV period across all ages using data from the National Notifiable Disease Surveillance System in Australia.²⁵ IRRs for ST3 increased in the <2 years (1.35), 2–4 years (4.65), 5–14 years (1.15). ST19A decreased across all paediatric age groups; <2 years (0.23), 2–4 years (0.25), 5–14 years (0.16). Similarly, ST1 was not detected in the <2-year and 2–4 year old groups; the IRR in the 5–14 years was 0.70. National surveillance of IPD in Australia consists primarily of serotyping *pneumococcus* cultured isolates; enhanced molecular testing of normally sterile sites is not routine. We therefore do not have PCR data for paediatric empyema in the interval between the two prospective cohort periods (from April 2009 to January 2015). We have previously demonstrated that enhanced molecular surveillance using PCR of empyema cases identified a ~sevenfold increase in ST3 and ST1 compared with routine serotyping of cultured pneumococcus isolates from pleural fluid;³⁴ this study further highlights the value for routine enhanced molecular surveillance.

In summary, the introduction of 13vPCV onto the Australian NIP was associated with a reduction in bacterial pneumonia hospitalisations but an increase in empyema hospitalisations in children. SP continues to be the main bacterial cause. The 13vPCV ST ST3 and 19A remain prevalent, with evidence that an increase in ST3 in particular, with a lesser contribution from non-13vPCV pneumococcus ST, may have driven a true increase in childhood empyema. The recent change in the Australian vaccination schedule to include a dose at 12 months may increase effectiveness of 13vPCV against ST3 and ST19A but

ongoing enhanced molecular surveillance will be required to monitor this given that the yield of identifying SP ST in pleural fluid is much higher with PCR compared with bacterial culture.

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Experiências adversas na infância e asma: trajetórias de uma coorte nacional

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RESUMO

Objetivo A investigação tem associado as primeiras experiências adversas da infância (EAI) ao desenvolvimento da asma. Contudo, os estudos existentes têm-se baseado geralmente na informação fornecida pelos pais sobre a exposição e os resultados. O nosso objetivo foi o de examinar a associação das EAI na fase inicial da vida com trajetórias empiricamente determinadas do risco de asma infantil, utilizando informação de registo independente tanto de exposições de resultados.

Métodos Com base nos registos nacionais, estabelecemos uma coorte de estudo de 466556 crianças nascidas na Dinamarca (1997-2004). Obtivemos informações sobre EAI durante os 2 primeiros anos de vida (luto, doença somática crônica parental e/ou doença mental parental) e diagnóstico de asma infantil ou uso de medicamentos desde o nascimento até aos 10 anos de idade nos Registos Nacionais Dinamarqueses de Doentes e Prescrições Médicas, respetivamente. Identificámos fenótipos de asma utilizando modelos de trajetória baseados em grupos. Utilizámos seguidamente a regressão logística multinomial para avaliar a associação entre as primeiras EAI e os fenótipos de asma.

Resultados Identificámos quatro fenótipos de asma: não asmática, transitória de início precoce, persistente de início precoce e asma de início tardio. Meninas com asma de início precoce transitória (OR 1,13, 95% IC 1,04 a 1,24), asma persistente de início precoce (1,27, 95% IC 1,08 a 1,48) ou asma de início tardio (OR 1,28, 95% IC 1,11 a 1,48) vs. não asmática eram mais suscetíveis de ter uma exposição precoce a EAI em comparação com meninas sem exposição a EAI. Os resultados foram semelhantes para os meninos que também sofreram EAI com um OR de 1,16 (95% IC 1,08 a 1,25), 1,34 (95% IC 1,20 a 1,51) e 1,11 (95% IC 0,98 a 1,25), respetivamente.

Conclusão Num estudo de população nacional, identificámos três fenótipos de asma na infância e descobrimos que as EAI no início de vida estiveram associadas ao aumento de probabilidade para cada um destes fenótipos de asma, tanto entre meninas como entre meninos.

INTRODUCTION

Asthma is the most common chronic disease in childhood and is associated with reduced quality of life and substantial direct and indirect societal costs.¹⁻³ Despite much research on the aetiology and risk factors, the development and progression of asthma is not well understood.⁴ Asthma is an umbrella term for multiple distinct pathophysiological mechanisms that vary in their clinical expression of disease. Given this heterogeneity, inconsistencies between previous studies investigating the incidence and aetiology of asthma are to be expected.⁵

Findings reported in the current literature indicate that adverse childhood experiences (ACEs) influence mental and physical health later in life.⁶⁻⁸ Experience of severe ACEs in childhood (eg, abuse, bereavement or neglect) has been linked with elevated rates of morbidity and mortality.⁹⁻¹⁴ Although the biological mechanisms linking ACEs with health outcomes later in life are undoubtedly multifaceted, recent research suggests dysregulation of the immune system may be one key underlying biological pathway.^{15,16} A few studies have examined the influence of ACEs

Key messages

What is the key question?

► What are the age-dependent asthma phenotypes in children, and are adverse childhood experiences early in life associated with increased risk of these asthma phenotypes?

What is the bottom line?

► Using group-based trajectory modelling, we identified four asthma phenotypes (nonasthmatic, early-onset transient, early-onset persistent and late-onset asthma), and found that adverse childhood experiences early in life were associated with increased odds of all three childhood asthma onset phenotypes.

Why read on?

► This is the first nationwide population-based study to characterise early childhood asthma trajectories. This study, furthermore, adds knowledge to the field of adverse childhood experiences and childhood asthma, using independent register data of both exposures and outcomes.

on later development of asthma.¹³ Their findings indicate strong associations, including significant dose-response relationships, but the majority of the studies suffer from methodological limitations, such as parental report of ACEs and asthma outcomes in their children, small sample sizes, cross-sectional designs and low response rates.^{13,14} We addressed a number of these limitations by leveraging a nationwide cohort to examine whether the experience of ACEs during early life (0-2 years of age) is associated with asthma among children followed up to the age of 10 years. We used a data-driven approach to examine trajectories of asthma phenotypes using Danish nationwide data in relation to ACEs. We hypothesised that exposure to ACEs would increase the risk of asthma. Prior research also suggests that both dose and type of ACE affect the association with asthma risk.¹³ Previous studies have also identified sex-differential effects, suggesting that girls may be more vulnerable to maternal stress post-natal, whereas boys may be more vulnerable to maternal stress during pregnancy.^{17,18} Given these previous findings, we also investigated dose dependency of ACE exposure relative to development of asthma and sex-specific associations, with the hypothesis that girls would be more vulnerable to ACEs than boys.

PATIENTS AND METHODS

Study subjects: study design

We used nationwide register data of live-born singletons in Denmark, born from 1 January 1997 to 31

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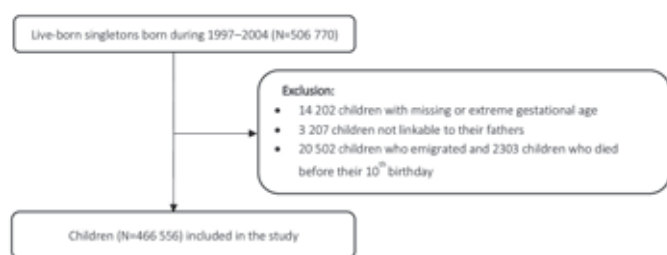


Figure 1 Flow chart illustrating the identification of the study population.

December 2004, and their parents and siblings (based on records of the mother), obtained from the Danish National Medical Birth Registry.¹⁹ A total of 466 556 children were included in this register based cohort study after exclusion of children with missing or extreme gestational ages (<154 or >315 days), children that could not be linked to their fathers, or children that emigrated or died before their 10th birthday (figure 1). Baseline characteristics of the nonincluded population are available in online supplemental table S1. We excluded children with missing data on gestational age or extreme gestational age because of the high risk of misclassifications as well as errors, obviously a result of typing errors in these administrative data, within these specific records of pregnancies. Baseline characteristics of this specific non-included population are available in online supplemental table S2. The large number of missing in this specific population supports a lower data quality. The children were followed from birth to ten years of age.

We linked the cohort to multiple nationwide administrative registers by the unique social security number assigned to all citizens in Denmark at birth.²⁰ Data from the Danish Civil Registration System, which contains information about sex, date of birth, identity of the parents, and emigration,²¹ was linked with:

- ▶ The Danish National Patient Register, which includes information on all hospital admissions (non-psychiatric) since 1977, and since 1995 also psychiatric inpatients, emergency department and outpatient contacts.²² For each patient contact since 1994, physician-assigned diagnoses are recorded according to the International Classification of Diseases, 10th revision (ICD-10).
- ▶ The Danish National Prescription Registry,²³ which includes information on all claimed prescriptions since 1995 from Danish pharmacies classified according to the Anatomical Therapeutic Chemical (ATC) classifications system.²⁴
- ▶ The Danish National Medical Birth Registry, which includes information pertaining to obstetrical outcomes and health of the new born.¹⁹
- ▶ The Population Education Register,²⁵ which includes educational level based on the International Standard Classification of Education (ISCED-2011).²⁶
- ▶ The Danish Register of Causes of Death, which records all deaths of citizens in Denmark according to cause of death based on ICD-10 codes.²⁷

ACEs early in life

We defined three categories of ACEs occurring in the first 2 years of life: (1) bereavement, that is, the death of a parent or sibling,²⁸ identified through the Danish Register of Causes of Death, (2) parental chronic somatic illnesses²⁹ based on the Charlson Comorbidity Index³⁰ but excluding diagnoses of asthma (ICD-10 codes: J45 and J46), using data from the National Patient Register and (3) parental mental illnesses²⁹ defined as any psychiatric diagnosis (ICD-10: F00–F99) as well as suicide attempts (ICD-10: X60–84 and Y87.0), obtained from the Danish National Patient Register. Multiple objective factors were chosen, as ‘stress’ is undoubtedly multifaceted. However, it has been speculated whether positive or negative events impact health later in life to a similar degree.⁵ Therefore, childhood events were operationalised as events that could be assumed to be negative with reasonable confidence.

Childhood asthma

Childhood asthma (age 0–10 years) was defined based on a diagnosis of asthma (ICD-10: J45) or status asthmaticus (ICD-10: J46), obtained from the Danish National Patient Register, or claims of at least

two prescriptions of any antiasthmatic medication within 1 year³¹ obtained from the Danish National Prescription Register. The ATC codes for inhaled asthma drugs were: inhaled b2-agonists (R03AC02–04, R03AC12 and R03AC13), inhaled glucocorticoids (R03BA01, R03BA02 and R03BA05), fixed-dose combination of inhaled b2-agonists and glucocorticoids (R03AK06 and R03AK07) and leukotriene receptor antagonists (R03DC03).^{23 24} The registers contain administrative data, and therefore the number of data points varies between individuals. We dichotomised the outcome into asthma/no-asthma yearly for each of the ten years, and each child, therefore, had 10 data points collected during the study period.

Statistical approach

We used Group-based Trajectory Modelling (GBTM)³² to identify clusters of children following similar progression for asthma development between birth and 10 years of age using the SAS PROC TRAJ macro.³³ The response variable used in estimation of the trajectories was yearly asthma status (yes/no). When fitting trajectory models, we iteratively changed the number of groups (3–6 group models) and shape of the trajectories and evaluated the Bayesian information criterion (BIC)³⁴ across models to select the model with the best fit (negative BIC closest to 0). We also ensured that distinct groups could be identified, as indicated by a $p < 0.05$. Based on previous findings,^{17,35} we a priori stratified the sample by sex of the child before fitting trajectories. We also examined the posterior probability of trajectory assignment to evaluate the quality of the classification further. We identified potential confounders based on prior literature and the use of Directed Acyclic Graphs (see online supplemental figure S1). The confounders included were: maternal age at delivery (<25/25–34/≥35 years), parental highest educational level at delivery (vocational education or below (ISCED level 0–3) or higher education (ISCED level 4–8)), maternal smoking during pregnancy (no/yes), maternal place of living at delivery (urban/rural), parity (nulliparous/multiparous), calendar year of birth (1997–1999/2000–2002/2003–2004) and season of birth (spring/summer/fall/winter).

We used multinomial logistic regression to examine sex-specific associations between ACEs and asthma phenotypes (childhood onset phenotypes vs never/infrequent asthma). We considered ACE scores in three ways: (1) any ACE vs none, (2) cumulative score ranging from 0 to 3 in which each category of ACE with at least one event was summed (ie, 0=no ACE in any category and 3=at least one ACE in each of the bereavement, somatic illness and mental illness categories) and (3) separate ACE-specific models (any bereavement vs none, any somatic illness vs none, any mental illness vs none).

In all multinomial models, we treated the non-asthmatic (NA) trajectory as the reference group. Statistical tests for interaction further justified our hypotheses by revealing $p < 0.0001$ of sex. To account for missing covariate data, we performed multiple imputations by chained equations using the fully conditional method using SAS PROC MIANALYZE. All statistical analyses were performed using SAS V.14.1 (SAS Institute).

RESULTS

A total of 3.8% and 3.9% of the girls and boys, respectively, were exposed to at least one ACE during the first 2 years of their life ACE-exposed children were slightly more likely to have either a younger (<25 years) or older (≥35 years) mother compared with non-exposed children (table 1). Furthermore, exposed children were more likely to have lower educated parents, be first-born, and their mothers were more likely to smoke during pregnancy. No differences were observed for maternal place of living at delivery, or season of birth.

While we identified that a five-group model had a slightly better fit as determined by BIC values compared with a four-group model, the difference was minor, thus we chose to use the four-group model (figure 2) to be consistent with earlier studies and improve comparability.^{5,36} Patterns were similar in girls and boys, although with slightly different proportions of children assigned to each trajectory. The four groups can be characterised as: (1) NA (girls=91%, boys=87%), (2) early-onset transient asthma (EOTA) (girls=5%, boys=8%), early-onset persistent asthma (EOPA) (girls=2%, boys=3%) and late-onset asthma (LOA) (girls=2%, boys=3%). The average posterior probability

Table 1 Baseline characteristics of girls and boys by exposed and non-exposed to ACEs early in life

Characteristic	Girls			P value*	Boys			P value*
	Exposed (N=8673)	Non-exposed (N=218 483)	Total (N=227 156)		Exposed (N=9257)	Non-exposed (N=230 143)	Total (N=239 400)	
Maternal age at delivery (years)								
<25	1624 (18.7)	30 994 (14.2)	32 618 (14.4)		1771 (19.1)	32 820 (14.3)	34 591 (14.4)	
25–34	5575 (64.3)	154 868 (70.9)	160 443 (70.6)		5831 (63.0)	162 856 (70.8)	168 687 (70.5)	
≥35	1474 (17.0)	32 621 (14.9)	34 095 (15.0)	<0.0001	1655 (17.9)	34 467 (15.0)	36 122 (15.1)	<0.0001
Parents highest educational level at conception								
Vocational education or below	5797 (66.8)	126 051 (57.7)	131 848 (58.0)		6150 (66.4)	132 562 (57.6)	138 712 (57.9)	
Higher education	2834 (32.7)	91 925 (42.1)	94 759 (41.7)		3060 (33.1)	97 016 (42.2)	100 076 (41.8)	
Missing	42 (0.5)	507 (0.2)	549 (0.2)	<0.0001	47 (0.5)	565 (0.2)	612 (0.3)	<0.0001
Maternal smoking during pregnancy								
No	4991 (57.5)	142 825 (65.4)	147 816 (65.1)		5482 (59.2)	150 299 (65.3)	155 781 (65.1)	
Yes	2315 (26.7)	37 719 (17.3)	40 034 (17.6)		2356 (25.5)	39 582 (17.2)	41 938 (17.5)	
Missing	1367 (15.8)	37 939 (17.4)	39 306 (17.3)	<0.0001	1419 (15.3)	40 262 (17.5)	41 681 (17.4)	<0.0001
Maternal place of living at delivery								
Rural	7678 (88.5)	194 375 (89.0)	202 053 (88.9)		8201 (88.6)	204 485 (88.9)	212 686 (88.8)	
Urban	981 (11.3)	23 895 (10.9)	24 876 (11.0)		1047 (11.3)	25 406 (11.0)	26 453 (11.0)	
Missing	14 (0.2)	213 (0.1)	227 (0.1)	0.2647	9 (0.1)	252 (0.1)	261 (0.1)	0.4169
Parity								
First	3475 (40.1)	93 633 (42.9)	97 108 (42.7)		3776 (40.8)	98 459 (42.8)	102 235 (42.7)	
≥2	5154 (59.4)	124 116 (56.8)	129 270 (56.9)		5445 (58.8)	130 850 (56.9)	136 295 (56.9)	
Missing	44 (0.5)	734 (0.3)	778 (0.3)	<0.0001	36 (0.4)	834 (0.4)	870 (0.4)	0.0002
Calendar year of birth								
1997–1999	2772 (32.0)	81 939 (37.5)	84 711 (37.3)		2922 (31.6)	86 465 (37.6)	89 387 (37.3)	
2000–2002	3556 (41.0)	82 300 (37.7)	85 856 (37.8)		3861 (41.7)	86 580 (37.6)	90 441 (37.8)	
2003–2004	2345 (27.0)	54 244 (24.8)	56 589 (24.9)	<0.0001	2474 (26.7)	57 098 (24.8)	59 572 (24.9)	<0.0001
Season of birth								
Winter	2138 (24.7)	56 040 (25.6)	58 178 (25.6)		2350 (25.4)	58 511 (25.4)	60 861 (25.4)	
Spring	2301 (26.5)	58 129 (26.6)	60 430 (26.6)		2375 (25.7)	61 390 (26.7)	63 765 (26.6)	
Summer	2116 (24.4)	53 727 (24.6)	55 843 (24.6)		2315 (25.0)	56 780 (24.7)	59 095 (24.7)	
Fall	2118 (24.4)	50 587 (23.2)	52 705 (23.2)	0.0272	2217 (23.9)	53 562 (23.3)	55 779 (23.3)	0.1186
Offspring asthma trajectories								
No asthma	7778 (89.7)	199 857 (91.5)	207 635 (91.4)		7788 (84.1)	199 479 (86.7)	207 267 (86.6)	
Early-onset transient asthma	532 (6.1)	11 208 (5.1)	11 740 (5.2)		871 (9.4)	17 937 (7.8)	18 808 (7.9)	
Early-onset persistent asthma	165 (1.9)	3346 (1.5)	3511 (1.5)		320 (3.5)	6140 (2.7)	6460 (2.7)	
Late-onset asthma	198 (2.3)	4072 (1.9)	4270.0 (1.9)	<0.0001	278 (3.0)	6587 (2.9)	6865 (2.9)	<0.0001

*The p values are computed using a χ^2 distribution.
ACE, adverse childhood experiences.

of group membership was 0.98 for girls and 0.97 for boys, indicating excellent classification of trajectory. Children assigned to the EOTA trajectory are characterised by an early-life asthma diagnosis or treatment around the age of 2 years, resolving by approximately 6 years of age. Odds of asthma among children assigned to the EOPA trajectory increases until approximately 4 years of age and persists throughout the remainder of the study period. In children assigned to the LOA trajectory, odds of asthma increases between four and 5 years of age and continues to increase in a consistent manner until approximately 9 years of age.

Adjusted analyses showed that any vs no ACE exposure increased the odds of EOTA for both girls (OR 1.13, 95% CI 1.04 to 1.24)

and boys (OR 1.16, 95% CI 1.08 to 1.25) and EOPA for both girls (OR 1.27, 95% CI 1.08 to 1.48) and boys (OR 1.34, 95% CI 1.20 to 1.51). Girls with any ACE also had higher odds of being assigned to the LOA group (OR 1.28, 95% CI 1.11 to 1.48). The direction of this association was similar in boys (OR 1.11, 95% CI 0.98 to 1.25) (figure 3)..

Subgroup analyses

Analysis of the early life ACE cumulative score in relation to asthma phenotypes did not indicate a dose–response association (table 2). Subgroup analysis of each ACE category in relation to asthma phenotypes (table 3) support the results of any ACE exposure.

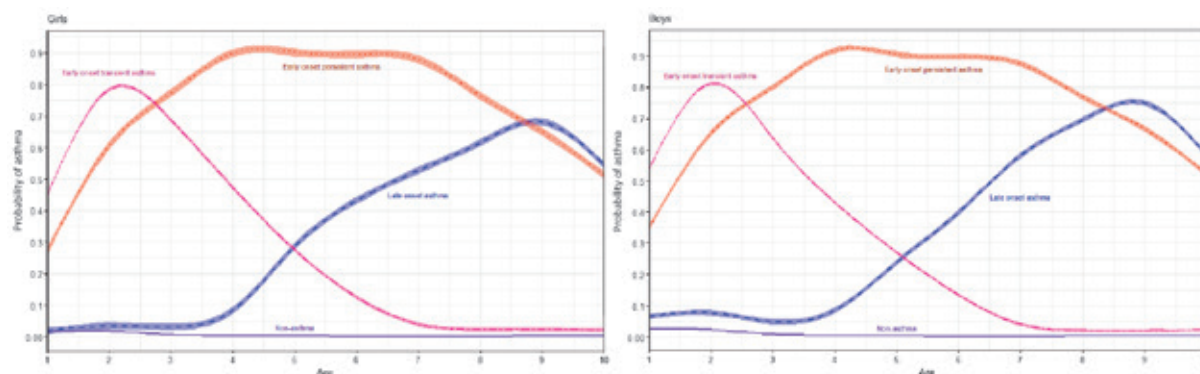


Figure 2 Phenotypes of asthma identified by group-based trajectories models using register data from a nationwide cohort of children born in Denmark between 1997 and 2004, by sex (girls, n=227 156, boys, n=239 400). Shaded area portray CIs of the trajectories.

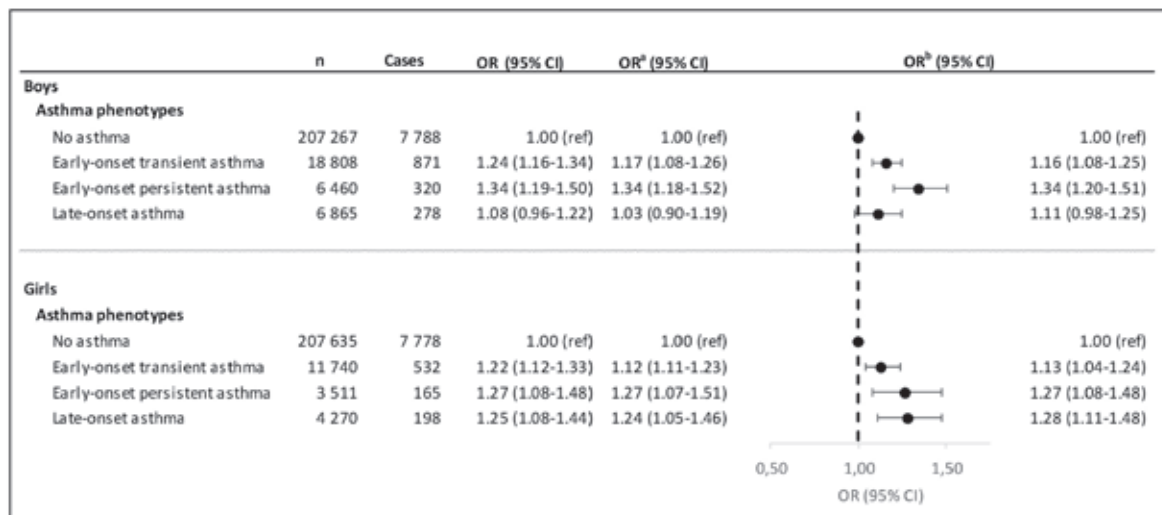


Figure 3 Results from sex-stratified multinomial logistic regression analyses between ACEs in early life (0/≥1) and asthma phenotypes. The population includes children born in Denmark between 1997 and 2004. ^aAdjusted for maternal age at delivery (years), parental highest educational level at conception, maternal smoking during pregnancy, maternal place of living at delivery, parity, calendar year of birth and season of birth. ^bIncludes adjustment and imputation by chained equations using a fully conditional method. ACEs, adverse childhood experiences.

DISCUSSION

In this nationwide cohort study of 466 556 children followed from birth to the age of 10 years, we identified four trajectories reflecting no/infrequent asthma and three asthma phenotypes among children. Notably, the phenotypes were similar for girls and boys, despite sex differences in the overall asthma prevalence. ACEs early in life were associated with an increased risk of asthma, which did not differ by the number or specific types of ACE. In general, no sex differences were observed in the associations between ACEs and asthma.

We used GBTM to distinguish empirically between trajectories of asthma, which is advantageous as it does not require the definition of asthma phenotypes by a predefined age of onset. To our knowledge, this is the first study based on a nationwide population to identify asthma phenotypes by sex. The average of the posterior probability of group membership was high for both girls and boys (close to 1), indicating that the trajectories were well defined and that misclassification of outcome was minimal. One published study identified similar trajectories in a population-based Canadian cohort with combined sex,⁵ and similar approaches have been used to identify wheezing phenotypes, which demonstrated comparable trajectories to ours.³⁶

The three asthma phenotypes identified by GBTM were similar for girls and boys, though with slightly different prevalence of asthma between sexes. Specifically, we found that more boys developed asthma before the age of 10 years compared with girls. This is in line with existing knowledge; for example, in the USA, the percentage of current asthma in boys <18 years is 9.2% vs 7.2% in girls.³⁷ Our results showed more pronounced associations among girls for LOA consistent with earlier studies.^{17,18}

Our data did not confirm a dose–response relationship as suggested by previous studies.³⁸ This might be due to a lack of power in our subgroup analyses, as only few children experienced more than one ACE. Our study used independent measures of exposures, outcomes and covariates. Furthermore, we included only events that most children would be assumed to perceive as adverse. While this provides a design that diminishes reporting bias, we were unable to capture the subjective experience of ACEs. Each specific category of ACE was associated with roughly a similar increase in risk of childhood asthma. A systematic review provides evidence that the impact of ACE on the development of asthma may be age-dependent.¹³ For example, a recent publication reported that postnatal maternal depression was associated with higher odds of asthma at the age of 6–8 years (an age span included in our study), but not after 9 years of age.³⁹

Methodological considerations

This study is based on a nationwide population, which minimises selection bias. Only 8% of the overall population was excluded. We did not see any large difference between the included and the excluded population with regard to the exposure (included girls 3.8% and boys 3.9%; excluded girls 3.9% and boys 4%), nor the outcome (included girls 8.6% and boys 13.5%; excluded girls 9.8% and boys 14.8%) (please see online supplemental table S1 for more details). We adjusted for a wide range of baseline characteristics but did not include potential confounding factors such as air pollution levels, or other environmental exposures (eg, parental occupational exposure implying a risk of exposing the children via a carrier home-effect) that might influence

Table 2 Results from sex-separated multinomial logistic regression analyses between cumulative ACEs in early life (0 vs 1, and 0 vs >1) and asthma phenotypes

Asthma phenotypes	Girls			Boys		
	n	Cases	OR* (95%CI)	n	Cases	OR* (95%CI)
Children who had experienced 1 vs 0 ACE						
No asthma	207 635	7413	1	207 267	7430	1
Early-onset transient asthma	11 740	508	1.12 (1.01 to 1.24)	18 808	832	1.17 (1.08 to 1.27)
Early-onset persistent asthma	3511	155	1.25 (1.05 to 1.50)	6460	306	1.34 (1.18 to 1.52)
Late-onset asthma	4270	190	1.24 (1.05 to 1.47)	6865	267	1.04 (0.90 to 1.20)
Children who had experienced >1 vs 0 ACE						
No asthma	207 635	365	1	207 267	358	1
Early-onset transient asthma	11 740	24	1.08 (0.69 to 1.69)	18 808	39	1.15 (0.81 to 1.64)
Early-onset persistent asthma	3511	10	1.77 (0.91 to 3.44)	6460	14	1.40 (0.80 to 2.44)
Late-onset asthma	4270	8	1.21 (0.57 to 2.56)	6865	11	0.96 (0.49 to 1.86)

The response variable is the asthma phenotypes, and no asthma is the reference. The population studied comprised of children born in Denmark between 1997 and 2004.

*Adjusted for maternal age at delivery (years), parents highest educational level at conception, maternal smoking during pregnancy, maternal place of living at delivery, parity & calendar year of birth and season of birth.

ACE, adverse childhood experiences.

Table 3 Results from sex-separated multinomial logistic regression analyses between specific early-life ACEs (bereavement/parental chronic diseases/parental mental illness or suicide attempt), each examined in a separate model and asthma phenotypes

Asthma phenotypes	n	Cases	OR* (95%CI)	Cases	n	OR* (95%CI)
Children exposed to bereavement (yes vs no)						
No asthma	207 635	417	1	456	207 267	1
Early-onset transient asthma	11 740	25	1.121 (1.01 to 1.23)	40	18 808	1.17 (1.08 to 1.26)
Early-onset persistent asthma	3511	7	1.27 (1.07 to 1.51)	15	6460	1.34 (1.18 to 1.52)
Late-onset asthma	4270	9	1.24 (1.05 to 1.46)	14	6865	1.03 (0.90 to 1.20)
Children exposed to parental chronic (yes vs no) diseases						
No asthma	207 635	3391	1	3995	207 267	1
Early-onset transient asthma	11 740	269	1.14 (0.99 to 1.31)	442	18 808	1.22 (1.09 to 1.36)
Early-onset persistent asthma	3511	88	1.37 (1.09 to 1.73)	176	6460	1.38 (1.17 to 1.64)
Late-onset asthma	4270	108	1.32 (1.06 to 1.65)	147	6865	1.04 (0.86 to 1.26)
Children exposed to parental mental illness or suicide attempt (yes vs no)						
No asthma	207 635	3601	1	3564	207 267	1
Early-onset transient asthma	11 740	255	1.09 (0.95 to 1.25)	412	18 808	1.14 (1.02 to 1.27)
Early-onset persistent asthma	3511	76	1.1920 (0.93 to 1.534)	138	6460	1.32 (1.09 to 1.57)
Late-onset asthma	4270	86	1.15 (0.901 to 1.46)	123	6865	1.02 (0.83 to 1.25)

The response variable is the asthma phenotypes, no asthma is the reference. The population studied comprised of children born in Denmark between 1997 and 2004. *Adjusted for maternal age at delivery (years), parents' highest educational level at conception, maternal smoking during pregnancy, maternal place of living at delivery, parity & calendar year of birth and season of birth.

ACEs, adverse childhood experiences

the associations.^{13–38} However, adjustment for various possible confounders, including maternal place of living at the birth of the child (urban/rural) as well as the highest educational level of the parents, did not substantially alter the associations.

However, we cannot rule out residual confounding. In addition, a limitation of the register-based studies is that we lacked information on some important confounders such as body mass index and environmental factors that could be related to both ACEs and asthma risk. Furthermore, some misclassification of both exposure and outcome cannot be ruled out.

ACEs early in life were obtained through registry data of death and morbidity, reflecting illness severe enough for the parents to seek professional help. This definition of ACEs most likely increases the specificity of ACEs, though it might also decrease in sensitivity, which is a limitation for register-based data. Ascertainment of asthma was based on a diagnosis of asthma or at least two claimed prescriptions of anti-asthmatic drugs. While healthcare services in Denmark are free of charge, prescription medicine is not, and any interpersonal differences in healthcare seeking behaviour, for example, due to economic restraint, might lead to misclassification of asthma phenotypes and likely result in estimates that are more conservative.

We measured ACEs between birth and 2 years of age. It is, therefore, possible that children assigned to the early-onset asthma trajectory experienced ACEs after asthma onset (reverse causation); however, it is unlikely that the included ACEs would occur because the child developed asthma. The mechanisms by which early life adverse events impact on asthma still needs further investigation.

CONCLUSION

In this large nationwide cohort study based on register data, three-childhood asthma onset phenotypes were identified, and each of these was more common in children experiencing adverse experiences early in life. Our results do not suggest marked differences in relation to child sex or differences with regard to the type or dose of ACEs.

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"Breathomics" para o clínico: a utilização de compostos orgânicos voláteis em doenças respiratórias

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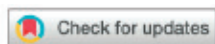
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RESUMO

A análise da respiração exalada tem o potencial de fornecer uma perspectiva valiosa sobre o estado das várias vias metabólicas que ocorrem nos pulmões localmente e noutros órgãos vitais, através da circulação sistêmica. Durante anos, os compostos orgânicos voláteis (COV) têm sido propostos como biomarcadores alternativos viáveis para diagnóstico e prognóstico de diferentes patologias respiratórias. Analisamos a literatura recentemente publicada sobre a descoberta de COV exalados e a sua utilização em várias doenças respiratórias. As principais barreiras no desenvolvimento de testes clínicos da respiração incluem a falta de consenso para a recolha e análise da respiração exalada e a complexidade de compreender a relação entre os COV exalados e as vias metabólicas subjacentes. Apresentamos uma visão geral abrangente, à luz da literatura publicada e da nossa experiência de coordenação de um centro nacional de "breathomics", dos progressos realizados até à data e de alguns dos principais desafios no terreno e das formas de os ultrapassar. Concentramo-nos particularmente na relevância de "breathomics" para os clínicos e nos conhecimentos valiosos que este acrescenta ao diagnóstico e monitorização da doença. "Breathomics" é uma grande promessa e as nossas descobertas merecem mais estudos de diagnóstico multicêntricos de larga escala, utilizando protocolos normalizados para ajudar a posicionar esta nova tecnologia no centro do diagnóstico de doenças respiratórias.

INTRODUCTION

Respiratory diseases remain among the leading causes of death worldwide.¹ By 2030, WHO estimates that respiratory illnesses will account for about one in five deaths worldwide.²

Early, rapid detection and treatment of lung diseases remain a priority, which would improve patient care and personalised therapy.³ For years, existing technologies like lung function tools and blood biomarkers have played an important role in diagnosing and monitoring lung diseases. However, there remains an unmet need for point-of-care respiratory-specific biomarkers that can aid in advancing precision medicine in both acute and stable respiratory diseases.

The lungs are almost unique owing to their ability to provide biological samples, direct from the organ with every breath. The ability to capture and analyse this sample type is highly attractive, as it allows direct non-invasive measurement of ongoing metabolic processes.

Breathomics, a branch of metabolomics studying exhaled breath, is a steadily evolving field that focuses on understanding the nature of volatile organic compounds (VOCs) and their health-related uses. VOCs can be leveraged as diagnostic biomarkers owing to their potential to mirror pathological processes taking place locally in the lungs and systemically, via the blood circulation.⁴ Additionally, they offer a non-invasive platform that is repeatable and potentially personalised, via 'breathprint' signatures.⁵ Despite years of clinical

trials, technical and statistical challenges have delayed further translation of this technology to a real-world clinical setting.

In this state-of-the-art review, we examine the current evidence, analytical challenges and future considerations of exhaled breath analysis in respiratory diseases.

Data sources and search criteria

For the purpose of this narrative review, a systematic search was conducted using the following evidence databases: (1) PubMed, (2) Medline and (3) EMBASE. The keywords and mesh terms used to complete the search included: 'asthma', 'volatile organic compound(s)', 'exhaled breath', 'VOC', 'VOCs', 'origin of VOCs', 'electronic nose', 'eNose', 'chronic obstructive pulmonary disease', 'respiratory infections', 'lung cancer', 'airflow limitation', 'Emphysema' and 'chronic bronchitis'.

Published peer-reviewed, full-text articles concerning clinical studies using VOCs in a diagnostic or disease monitoring capacity were assessed for eligibility. The following study types were included: observational studies, cross-sectional, case-control and cohort, and randomised controlled trials. The references lists of included studies were scrutinised to identify further relevant studies.

The studies were assessed based on their methodology and published results. Key findings from these studies are presented in the relevant sections.

Historical perspective of breath analysis

Utilisation of exhaled breath VOCs for disease diagnostics dates back to ancient Greek civilisations where breath was used to diagnose various illnesses. For example, the fruity smell of diabetic ketoacidosis and the fishy smell of liver illnesses.⁶⁻⁸ These elementary smell detection tests can be considered as the foundation of breath analysis.

The 20th century witnessed remarkable achievements in the field of breath testing, notably in 1971 Nobel Prize winner Linus Pauling presented a gas chromatogram showing separation of volatile substances from human breath, subsequently describing 250 components in exhaled breath.⁹ It was not, however, until the mid-eighties when Gordon *et al* demonstrated the feasibility of analysing exhaled breath VOCs in early diagnosis of lung cancer.¹⁰ This early association of VOCs and human disease formed the foundation for the current use of breathomics in early diagnosis and stratification of lung diseases.

In the following years, exhaled breath analysis gained increasing attention as a tool for diagnosing various illnesses. The specific pathways for these VOCs are not fully understood, nonetheless, profound progress has taken place with analytical technologies and detection capabilities.

Volatile organic compounds

Each exhaled breath contains thousands of VOCs; a heterogeneous group of carbon-based chemicals

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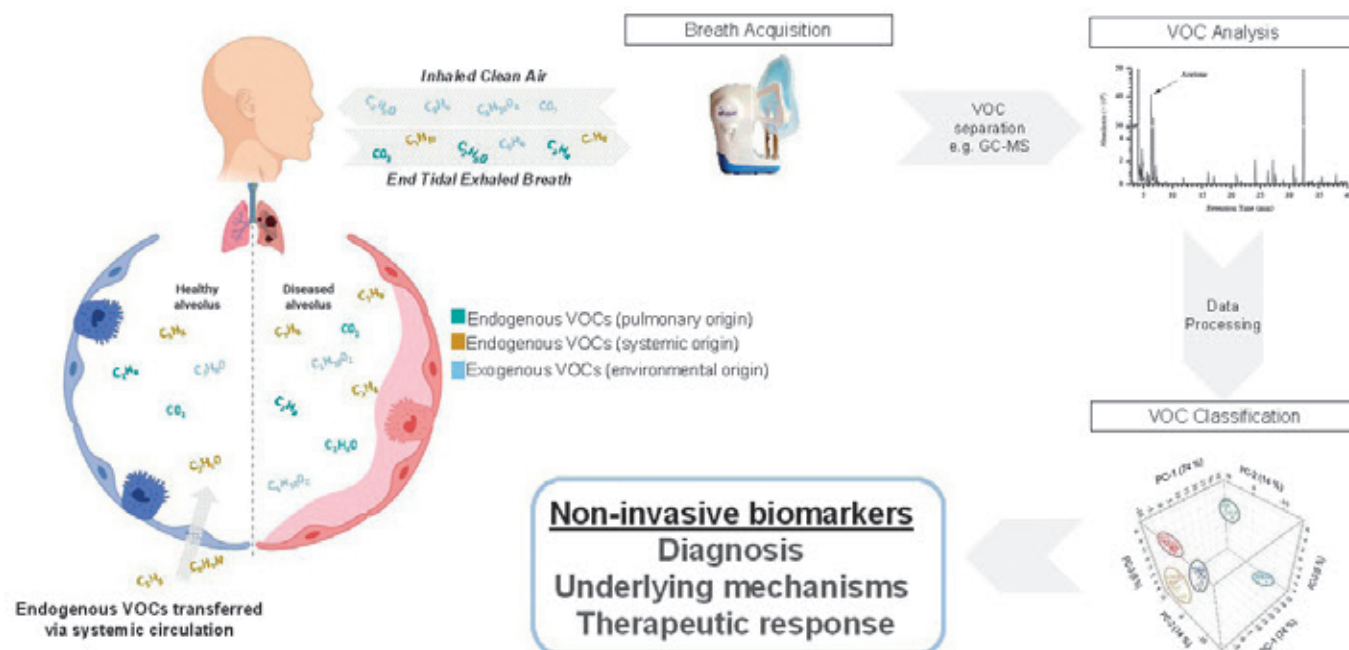


Figure 1 This figure highlights the complex kinetic of gaseous exchange. Endogenous VOCs can originate from the lungs or distant organs, via systemic circulation. Exogenous VOCs are continuously introduced into the respiratory system which can result in the production of volatile downstream products. Breath samples containing endogenous and exogenous VOCs are analysed to generate clinically meaningful data. GC-MS, gas chromatography mass spectrometry; VOCs, volatile organic compounds.

characterised by a high vapour pressure resulting from a low boiling point at room temperature.

Each breath cycle consists of different breath phases, and breath samples are often captured from the phase involved in gaseous exchange. This is also known as the 'end expiratory phase' or 'alveolar breath', excluding the dead space.^{11,12} This can be achieved using 'gated sampling', a process by which fractions of breath are collected based on measured parameters.

VOCs can originate from the external environment (exogenous) or from internal metabolic processes (endogenous) (figure 1). The presence of abundant exogenous compounds in breath samples (ie, environmental contamination) represents a fundamental challenge in breath research. Exogenous VOCs are continuously introduced into the respiratory system and owing to the complex kinetics of gas exchange, these can result in the production of volatile by-products, via various interactions with airway microbiota and mucosal lining.¹³

Removal of exogenous VOCs may simplify analysis, but loses potentially useful signals and requires additional processing steps. For example, limonene, a widely used food additive and fragrance for cosmetic or cleaning products,¹⁴ is present in higher levels in patients with liver cirrhosis and those with hepatic encephalopathy symptoms.¹⁵

In essence, exogenous VOCs and environmental contamination should be given special consideration when analysing exhaled breath VOCs for discovery studies; it continues to be an area of great uncertainty and larger multicentre studies validating environmental exposomes should be carried out.

Breath collection and storage

VOCs are found in trace levels (mainly in parts per trillion to parts per billion range) which poses considerable analytical challenge to operators.¹⁶ Current technologies allow for hundreds of VOCs to be detected in each exhaled breath sample. Breath collection is a key step in this process and sub-optimal sampling can introduce contaminants, lose potential markers or alter the balance of breath patterns. As a result, considerable effort has been put into improving and standardising sampling and preconcentration steps.

Breath sampling can either be direct, usually with point-of-care analysis, known as 'online' sampling; or indirect, with breath stored for lab-based analysis, known as 'offline' sampling. In both, careful attention needs to be paid to the choice of sampling process and analytic platform.

Collection bags made of Tedlar, polytetrafluoroethylene (PTFE) or foil have been widely used as receptacles for breath sample storage. Bags are attractive as they are a convenient, inexpensive and are disposable for potentially infectious samples,¹⁷ however, potential drawbacks are (1) compound degradation within collection bags, particularly when samples remain mixed with water vapour and (2) compound interactions within the bag product. Additionally, Steeghs *et al*¹⁸ tested the compatibility of Tedlar bags and highlighted two abundant compounds contaminating bag contents. A reproducible compound loss was also detected both during bag filling and at a later stage following storage. Important considerations and suggestions for bag handling have been published.¹⁷

Various direct breath collection devices have emerged over the last few years.^{19–21} One example is the Respiration Collector for In Vitro Analysis breath sampler (Owlstone Medical, Cambridge, UK), which is a handheld, portable device, designed to collect breath directly onto sorbent tubes that are then transferred for analysis.²² The portability of such devices allow for breath collection at the patient's bedside.

Sorbent tubes are commonly used for trapping and transporting VOCs from breath samplers to analytical devices, offering significant cost and logistical advantages.²³ Sampling onto sorbent tubes is usually carried out using a calibrated pump where air is drawn through the tube at a constant rate and as the breath sample passes through the tube, compounds are collected on the absorbent inside.

A common concern with this method is that sorbents can retain moisture, given the high water vapour content in breath, which can negatively affect the quantitative capture of some analytes. In an attempt to overcome this problem, samples can be dry purged where a pure inert gas is passed through the sorbent tube to eliminate any additional trapped moisture while retaining analytes.²⁴

VOCs are released from the tube for analysis using a process known as thermal desorption. Samples are heated to allow for sample extraction from the absorbent interior onto a pre-cooled trap, before further desorption into the analytical system. This offers numerous benefits including concentration enhancements, amplifying detection limits and eliminating unwanted analytical interferences.²⁵

Other considerations when undergoing breath collection include the time of day. Wilkinson *et al*²⁶ demonstrated a circadian variability in a proportion of exhaled VOCs over a 24-hour period with differential patterns of VOC release in asthmatics compared with healthy breath.

Breath analysis

There are a number of technologies that can be used to analyse breath samples. Broadly these can be divided based on offline and online sampling techniques (figure 2).

Offline technologies

Offline technologies are considered the gold standard techniques for breath analysis and include:

Gas chromatography mass spectrometry (GC-MS) is the most common chromatographic technique, allowing for compound separation and identification based on both retention time and mass spectra matching.²⁷ Gas chromatography comprises two main phases (1) the mobile phase: the vaporised sample is carried in an inert gas (eg, helium) at a predetermined speed which is then passed through a chromatographic column and (2) the stationary phase: compounds are separated based on the strength of interaction between the molecules and the column; with the time taken for it to pass through the column known as retention time. Despite being highly sensitive and reproducible, complex presample processing, prolonged analysis time and expert knowledge requirement has hampered its use in a wider clinical setting. A number of studies have emerged over the years using GC-MS to examine specific VOCs for lung pathologies,^{28–30} however, the lack of standardisation and methodological platforms limited further exploration of this technology in wider multicentre comparison studies.

Comprehensive two-dimensional GC-MS (GC × GCMS) is a multidimensional gas chromatography technique where the addition of an extra column provides superior separation over conventional GC-MS.³¹ As the analytes elute from the primary column, they are modulated onto a secondary column. This shorter secondary column leads to further separation allowing peaks with similar volatility, which could not be separated adequately with one-dimensional chromatography, to be separated by another mechanism. This is particularly helpful in complex matrices like breath samples.^{31–33} As the technique is more advanced, it has a higher initial capital cost compared with traditional GC-MS and requires more specialist skills to operate.

Online technologies

Online technologies involve direct introduction of breath samples into analytical instruments for analysis, negating the need for sample storage. As online technologies often require less analytical time results can be obtained immediately and, owing to their portability, they offer a potential for point of care testing. However, this means any chromatographic separation and analytical detector are often simplified,

reducing the ability to identify compounds with high fidelity. This, and the lack of data processing parameters, limits its applications to proof-of-concept benchmarking studies and validation studies.³⁴

Common examples of these technologies include:

GC ion mobility spectrometry

First described by McDaniel in the 1950s, ion mobility spectrometry (IMS) is an analytical technique that separates and identifies ionised molecules in the gas phase based on their mobility in a carrier buffer gas,³⁵ it can detect VOCs down to ultratrace levels (ng/l to pg/l range) without the need for pre-concentration, visualising VOCs in a 3D IMS chromatogram. Without identifying individual chemical components, IMS recognises peak patterns that can be used for disease recognition. Its simplicity and easy patient interface allowed its utilisation in few studies in the last decade.^{36–39}

Proton-transfer-reaction mass spectrometry (PTR-MS): PTR-MS has the capability of real-time analysis: it is considered one of the fastest analytical techniques with a typical time resolution of <100ms. VOCs are ionised by transferring a proton from the reagent ion, hydronium, to any molecules with a suitable proton affinity, which are then separated in the mass spectrometer. Despite its speed the lack of pre-concentration can limit sensitivity and the absence of chromatographic separation limits its ability to definitively identify compounds compared with GC-MS.

Electronic nose technology

Loosely mimicking human olfaction, electronic noses (eNose) are made of multiple array sensors programmed to recognise different odours and comparing them to preprogrammed patterns.⁴⁰ Array sensors convert chemical input (breath samples) into electrical signals.⁴¹ eNoses do not contribute to individual compound identification, instead disease separation occurs through recognition of different breath profiles, also known as 'breath prints' or 'breath signatures' using pattern recognition algorithms.

Unlike GC-MS, analysis eNoses do not require highly skilled operators, and has a relatively quick operational time (results within minutes), with lower technical costs. Its readily implementable nature makes it more suited for point of care clinical testing compared with other offline technologies.⁷ However, there are some disadvantages compared with mass spectroscopy, mainly the inability to identify named compounds in complex mixtures, making it impossible to link back to metabolic processes and mechanistic pathways.⁴² Additionally, the breath signatures are highly influenced by environmental factors and water vapour, so considered to be less rigorous.⁷

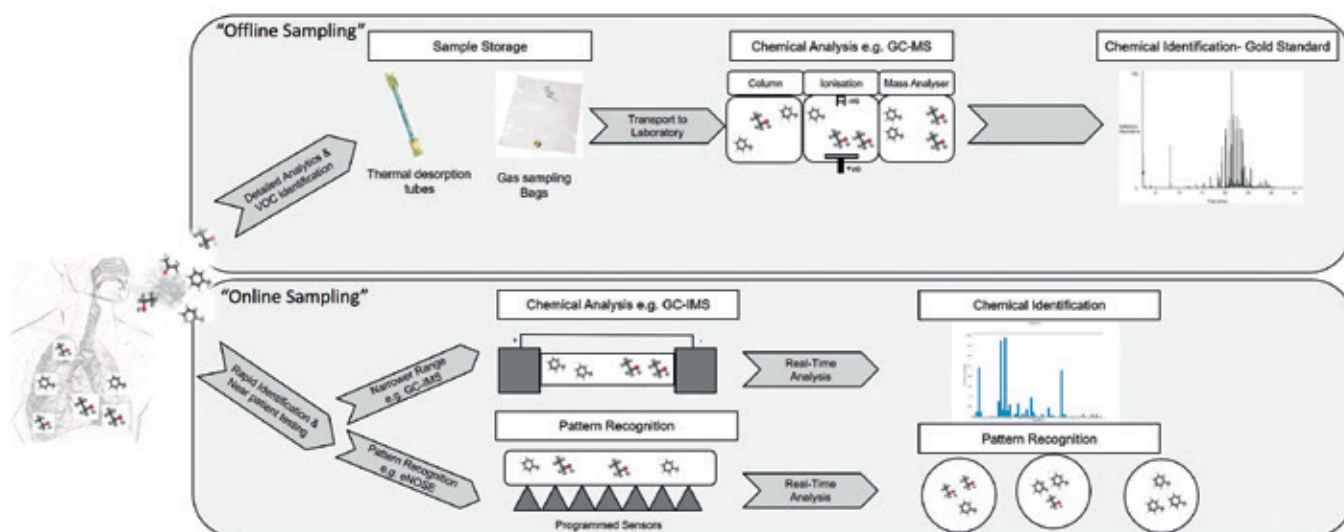


Figure 2 Exhaled breath VOCs can be analysed using offline or online technologies. Offline technologies, currently considered gold standard, involve storing samples in a sorbent tube or collection bag prior to injecting them to an analytical instrument (eg, GC-MS). Online technologies involve direct introduction of breath samples to analytical instruments for analysis, negating the need for sample collection and storage. Online technologies require less analytical instrument time and technical skills and results can be obtained immediately, however, they lack the ability to identify compounds with high fidelity which limited its applications. GC-MS, gas chromatography mass spectrometry; VOCs, volatile organic compounds.

Several diagnostic studies have been carried out using eNoses in airway disease^{43–46} and lung cancer^{47–49} with good discriminatory power. Furthermore, Plaza *et al*⁵⁰ described the ability of breath signatures in stratifying different phenotypes of asthma based on their sputum granulocytic count.

As described, eNose technology has the potential to make a powerful screening tool for various pulmonary diseases. Further largescale pragmatic clinical trials are required to further validate this. The limited sensor stability, inability to calibrate and the difficulty in mass generating identical sensors have hindered further translation of this technology to a real-world clinical setting.

Headspace analysis

Headspace refers to the volume of gas directly above and in contact with a biological sample. Headspace has been used as a VOC source for a number of solid and liquid samples. For headspace analysis purposes, samples are usually kept within sealed glass vials that are either heated or air is driven over them to stimulate VOC release out of samples. Once stabilised, the gas within the vial is then collected or directly transferred to instruments for analysis.

Although still in the early stages of development, headspace analysis has been used to investigate compounds from bacteria implicated in ventilator-associated pneumonia⁵¹ and the identification of more specific organisms such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus fumigatus*,^{52–54} with promising results.

In vitro breath analysis adds to the growing body of evidence supporting the use of headspace VOC analysis in clinical practice, however, it faces many challenges including sample degradation requiring standardised protocols for sample storage and treatment.

VOCs in respiratory disease

Exhaled breath of healthy individuals contains a wide range of VOCs at varying concentrations. These compounds include, but are not limited to, hydrocarbons, ketones, aldehydes and alcohols.⁵⁵ A breakdown of the various functional groups and their structure formulas are highlighted in (online supplemental table 1). The content and concentrations of these VOCs vary depending on the underlying metabolomic pathways during health and disease states as well as environmental interferences.

It was not until advanced analytical techniques were introduced in the 1990s that a complete set of human breath profile had emerged for the first time.⁵⁶ Hydrocarbons were one of the first discovered compounds in human breath, dating back to 1963 Ram Chandra and Spencer reported unexpected ethylene levels in exhaled human breath that were not thought to be solely attributable to gut flora.⁵⁷ This was later believed to be associated to disease state when small chain hydrocarbons, which were thought to be a direct result of lipid peroxidation, were identified in exhaled breath.⁵⁸

Exhaled breath VOCs analysis has been utilised in a variety of respiratory conditions, including:

Airway diseases

Asthma and chronic obstructive pulmonary disease (COPD) are two of the most common respiratory diseases affecting millions of people.⁵⁹ The utilisation of VOCs in airways disease is promising, although to date there are no definitive diagnostic breath signatures for either disease to aid disease classification. Numerous studies have evaluated the use of exhaled VOCs in diagnosing and phenotyping airways diseases, with the most commonly identified compounds belonging to carbonyl-containing groups (ie, aldehydes, esters and ketones) and hydrocarbons (ie, alkanes, alkenes and monoaromatics).^{60–62}

In one of the largest exhaled breath studies in asthma, Schleich *et al*⁶³ were able to successfully classify 521 asthmatic patients into three groups based on their sputum granulocytic cell count, potentially offering surrogate biomarkers for eosinophilic and neutrophilic asthma.

Exhaled breath VOCs have been shown to successfully separate asthma and COPD,^{64,65} breath signatures using eNoses have been shown to do the same based on clinical and inflammatory characteristics rather than disease diagnosis.⁶⁶

Basanta *et al*⁶⁷ investigated the relationship between exhaled breath VOCs and existing indices of inflammation and described in great detail the ability of GC-ToF-MS in discriminating patients with COPD based on inflammatory cells into eosinophilic and neutrophilic subgroups, this particularly relevant in precision medicine and assessment of treatment response.

Exhaled breath analysis shows a promise in enhancing our knowledge of the pathogenic pathways driving airway diseases. The use of VOCs as stratification biomarkers in this diverse patient population has the potential to transform the care we offer. Further progression towards a real-world clinical translation will highly depend on the implementation of large-scale, well-powered, multicentre clinical studies.

VOCs in respiratory infections

The treatment of microbial respiratory infections is an obvious target for breathomics, as early and accurate identification of causative organisms can be challenging, particularly in patients with severe infections.⁶⁸ Micro-organisms produce a wide variety of volatile metabolites, which can be released in the stable state or when the cell is disrupted in cases of infection;^{69–71} these volatiles can serve as a biological marker of microbial presence and have the potential to enhance the diagnostic process, improving clinical outcomes.⁷²

The presence of distinct VOC profiles in pneumonia has been demonstrated by multiple studies,^{73–75} however, none have established sufficient granularity to accurately diagnose pneumonia based on a single breath test. A systematic review by van Oort *et al*⁷⁶ outlined nitric oxide (NO), among others, as a potential diagnostic biomarker; though this is thought to be less specific as various other respiratory conditions drive altered NO bioactivity during disease state.⁷⁷

Boots *et al*⁷⁸ examined two hundred samples of bacterial headspace (defined as the area of gas directly surrounding a sample) from four different microorganisms (*E. coli*, *P. aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*) and demonstrated a highly significant difference in VOC occurrence of different bacterial cultures. Additionally, they demonstrated separation between methicillin-resistant and methicillin-sensitive isolates of *S. aureus* potentially translating to a valuable diagnostic tool in medical microbiology.

There is an urgent unmet need for a rapid and accurate test to diagnose tuberculosis, owing to the high diagnostic delay.^{79,80}

Breath analysis has the potential to diagnose TB with moderate accuracy⁸¹ through the detection of specific VOCs produced by *Mycobacteria*,^{82,83} however, implementing VOCs as standard diagnostic tools will require further developments.

Cystic fibrosis (CF) is a growing area of interest in respiratory medicine, several studies have examined the role of exhaled breath VOCs in CF patients; Kramer *et al*⁸⁴ demonstrated a proof-of-concept approach to using exhaled VOCs for the rapid identification of infectious agents in CF patients with lower respiratory tract infections.

The 2-aminoacetophenone (2-AA) was assessed for its specificity to *P. aeruginosa* in 29 CF patients and its suitability as a potential breath biomarker using GC-MS.⁸⁵ The 2-AA was detected in a significantly higher proportion of subjects colonised with *P. aeruginosa* (93.7%) than both the healthy controls (29%) and CF patients not colonised with *P. aeruginosa* (30.7%) indicating that (2-AA) is potentially a promising breath biomarker for colonisation.

Breath analysis has the potential to be positioned in both the diagnostic and therapeutic work flows of respiratory infections, guiding early diagnosis and judicious antimicrobial use.

VOCs in lung cancer

Lung cancer has a poor prognosis, mostly due to the lack of symptoms and late presentation. While screening with CT has been introduced, the ability to diagnose through breath would likely lead to significant clinical impact, with considerably less radiation exposure to patients.⁸⁶

Metabolic changes within cancer cells can lead to significant changes in volatile breath profile.⁸⁷ Over the years, this has been

explored as a potential avenue for early detection and diagnosis of lung cancer.^{88–91}

One of the first studies to use VOCs in lung cancer was carried out by Gordon *et al.*¹⁰, they reported a GC-MS profile of exhaled breath profile of 12 samples from lung cancer patients and 17 control samples with almost complete differentiation between the two groups.

Bajtarevic *et al.*⁸⁹ expanded on this to include an additional analytical instrument, using both PTR-MS and solid phase microextraction with subsequent GCMS, with a larger sample size (220 lung cancer patients at different stages of illness and 441 healthy volunteers), they reported that the three main compounds appearing in everybody's exhaled breath (isoprene, acetone and methanol) were found at a slightly lower concentration in lung cancer patients compared with healthy volunteers using PTR-MS, additionally, the sensitivity of detection of lung cancer volatiles in breath based on the presence of four different compounds was only 52%, going up to 71% when including 15 compounds. The compounds identified were mainly alcohols, aldehydes, ketones and hydrocarbons.

Dragonieri *et al.*⁴⁸ adopted a different approach by using eNose and was able to discriminate breath profiles of 30 subjects with non-small cell lung cancer from patients with COPD and healthy volunteers, with modest accuracy.

The aforementioned studies have formed the foundation for large scale clinical trials evaluating the use of exhaled breath VOCs in patients with a clinical suspicion of lung cancer.⁹² Further results of large scale trials are eagerly anticipated.

VOCs in other respiratory conditions

Exhaled breath VOCs have been used in various other respiratory illnesses, nearly 90% of 25 patients with *Pneumocystis* were discriminated by their breath profile (receiver operator characteristic area under the curve (ROC-AUC) 0.88).⁹³

Several studies examined the role of exhaled VOCs in interstitial lung disease (ILD), Yamada *et al.*³⁹ described five characteristic VOCs in the exhaled breath of IPF patients, using multicapillary column IMS. Of the five VOCs, four were identified as p-cymene, acetoin, isoprene and ethylbenzene. Further work in ILD was carried out using IMS, which seems to be a promising technique in discriminating patients with ILD from healthy controls.⁹⁴ eNose sensors were used in patients with obstructive sleep apnoea, breath prints changed dramatically after a single night continuous positive airways pressure (CPAP) and changes conformed to two well-distinguished patterns indicating that exhaled breath prints can potentially qualify as a surrogate index of response to and compliance with CPAP.⁹⁵

The most clinically relevant volatile compounds are listed in (online supplemental table 2) along with the corresponding analytical technologies and reported concentration changes.

Clinical implementation

The non-invasive nature, low patient burden and ability to directly sample from the target organ, makes the adoption of breathomics in real-world clinical practice an attractive prospect. However, clinical implementation has proved more challenging. The majority of reported breath studies so far have been small in size, single-centred and with no external validation. Nonetheless, several papers have assessed the feasibility of breath sampling in various clinical settings, including outpatient clinics,^{61,96} acute admission units,^{97,98} and intensive care units,^{74,99} with promising results.

External validation of breath biomarkers in independent populations is considered instrumental as it produces reliable predictions that can be reproduced in other clinical settings. The lack of external validation has created significant reporting challenges. From our review, there is little overlap between biomarkers reported by various groups which can be partially explained by the differences in methodology and reporting tools. The first step towards establishing a breathomics platform in clinical settings would be to regulate practices, including agreed common standardised operating procedures for breath collection, storage and analysis.

Clinical implementation of breathomics is thought to be particularly relevant amidst the ongoing COVID-19 pandemic. SARS-CoV-2 infection has been reported to cause a multitude of symptoms that affect several organs and systematic metabolism resulting in altered volatile metabolite distribution. Additionally, the rapid detection of COVID-19 specific VOC panel is thought to be particularly rewarding if tuned to assess the negative predictive value; this can be used to screen large populations (eg, airports, schools) as a first line test in ruling-out rather than ruling-in test, and to determine which individuals need further testing. This will enable rapid decision making as well as provide complementary information that will strengthen disease diagnostics.

Challenges and future considerations

Current analytical technologies have demonstrated an innovative ability to separate and detect a wide range of exhaled breath VOCs, however, the implementation of these techniques in a real world clinical setting faces considerable chemical and analytical barriers. One of the major unresolved challenges is that of environmental contamination and their interference with exhaled breath VOCs, there is still no unified consensus on how to tackle this issue; as relevant as it is to subtract environmental VOCs,¹⁰⁰ it is crucial to take all molecular breath interactions into consideration when generating a diagnostic breath matrix. Standardised protocols should be instated for breath collection, analysis and reporting to guide future studies and allow a transparent analytical comparability across sites.

The availability of multiple analytical platforms with contrasting performance measures adds to the complexity of standardising biomarker discovery protocols. The choice of technology comes down to device availability and study budget, however, discovery studies are carried out using devices with the ability to separate and detect compounds with higher sensitivity and established robustness like (GC-MS). Although considered gold standard, GC-MS devices are considered highly complex for the non-experienced and are time consuming, making them less desirable in a real-world clinical setting. Sensor array-based technologies are much easier to use but are usually spared for when studies are aiming to distinguish between breath profile without the need for named compounds.

Analysis of breath matrices is highly complex, the combination of large variables and a relatively small sample size has led to various analytical challenges, the most common being that of 'overfitting'. With overfitting, usually owing to a limited sample size, the whole dataset is used to train and validate discovery prediction models, as opposed to having separate discovery and replication datasets, this results in a falsely optimistic models that can't be generalised to the entire population. Ideally, this is overcome by training prediction models in a distinct dataset that is separate to and independent from the validation dataset, currently considered the gold standard method.^{101,102}

Exhaled breath biomarkers are envisaged to have a crucial role as point-of-care tests in emergency departments and primary care clinics, however, to our knowledge no major studies have been completed in these settings. To date, biomarker discovery studies have mostly been small in size and confined to single centres. With few exceptions,^{103–105} the majority of the published breath discovery studies have been carried out in the stable disease state or at an outpatient clinic level. Further large-scale trials targeting acute disease states are required to properly evaluate the reliability of these breath tests and to formally assess the replicability in a real-world clinical setting.

Exhaled VOCs can provide valuable insight into the metabolic processes in the human body beyond the lungs, this can further expand our understanding of the common respiratory metabolic traits, translating into improved patient-centred diagnostics and therapeutic measures. Only when the aforementioned challenges are addressed, can the value of breath technology be fully appreciated.

Conclusion

Exhaled breath analysis possesses an inherent appeal that has been explored by scientists and clinicians for many decades. The lack

of consistency in trial outcomes among other challenges have hindered faster translation of this technology into a real-world clinical setting. Considerable effort has been invested over the last few years to address these issues but exhaled breath analysis is still far from clinical implementation. In this state-of-the-art review, we presented a comprehensive critique of the published literature and highlighted some of the key challenges and ways to overcome them.

Looking at the current state of the field compared with where it was 10 years ago predicts an encouraging future for exhaled breath analysis that can potentially revolutionise healthcare and point-of-care diagnostics.

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Arrefecer a controvérsia acesa de um cigarro mais seguro: o calor sem combustão não é melhor do que os cigarros de combustão tradicionais

Irina Petrache,^{1,2} Esther de Boer²

Em resposta a provas esmagadoras dos efeitos nocivos do tabagismo, a indústria do tabaco lançou os cigarros sem combustão (HNBC), um híbrido entre cigarros tradicionais de combustão (TCC) e dispositivos eletrônicos de vaping. Comercializaram o HNBC como uma alternativa menos nociva ao TCC devido à ausência de tóxicos específicos libertados pela combustão do tabaco. No entanto, à semelhança do fumo de TCC, os aerossóis libertados pelo aquecimento do tabaco (até 350°C) em HNBC contêm nicotina e elementos nocivos gerados pela combustão incompleta (pirólise) e degradação termogénica do tabaco. As alegações de que estes subprodutos estão presentes em quantidades demasiado pequenas para serem prejudiciais sugerem que existe uma concentração limite para os efeitos tóxicos dos subprodutos do tabaco aquecido. Esta afirmação requer uma maior elucidação, devido a uma escassez de estudos sobre os efeitos a longo prazo do HNBC na saúde e ao uso crescente do HNBC, especialmente entre os fumadores actuais de TCC.¹ Por conseguinte, os dois relatórios da edição original da Thorax número de junho de 2021, de Loffredo *et al* e Kanai *et al*,^{2,3} dois grupos de investigação sem ligação com a indústria do tabaco, são oportunos, fornecendo novos conhecimentos sobre o impacto prejudicial do uso crónico de HNBC na função endotelial e questionando a sua utilidade para a cessação do tabaco, respectivamente.

O presente relatório, *SUR-VAPES Chronic Study* de Loffredo *et al* é um seguimento do seu estudo anterior, *SUR-VAPES 2 Randomised Trial*,⁴ que encontrou efeitos agudos menos prejudiciais na função endotelial do HNBC quando comparado com TCC e *vaping*. O optimismo inicial sobre o HNBC que se seguiu foi temperado por um relatório de um grupo diferente,⁵ que mostrou efeitos

agudos prejudiciais do HNBC no sistema cardiovascular de magnitude semelhante ao TCC. Agora, o Estudo Crónico SUR-VAPES mostra, pela primeira vez, que o uso crónico de HNBC tem efeitos prejudiciais sobre a função endotelial semelhantes aos do TCC. Apesar das limitações do estudo, que incluem a falta de aleatorização, diferenças de base na idade e duração do uso do tabaco entre grupos e dependência da auto-notificação, estes resultados são biologicamente plausíveis, dados os efeitos tóxicos da nicotina por si só no endotélio⁶ e apoiam a afirmação das autoridades de saúde de que a substituição do tabaco de combustão por outros produtos pode não ser mais segura.

Na sua tentativa de responder a outra questão importante, se o HNBC pode ajudar como ponte para a cessação do tabagismo, Kanai *et al* estudaram indivíduos saudáveis num local de trabalho japonês. Relataram que os utilizadores de HNBC apresentaram menos probabilidades de cessar o consumo de tabaco do que aqueles que utilizavam exclusivamente TCC, e que uma percepção de danos menores e a residência com outros utilizadores de HNBC estavam associadas à persistência do consumo de tabaco. Apesar do seu pequeno tamanho de amostra com reduzida generalização e uma avaliação limitada da cessação, incluindo detalhes escassos do uso de HNBC, o estudo tem uma mensagem impactante. Salienta os efeitos prejudiciais da promoção de HNBC como uma alternativa mais segura ao TCC relativamente aos esforços de cessação. Adicionalmente, os seus resultados são apoiados por uma análise secundária anterior⁷ que mostrou, numa coorte distinta, a falta de associação do HNBC com a cessação de TCC, negando a utilidade geral do uso de HNBC na cessação do tabagismo. Os relatórios de Loffredo *et al* e Kanai *et al* dão ímpeto à realização de estudos de validação aleatórios de maior dimensão e à avaliação do impacto do HNBC em parâmetros de saúde adicionais. O seu trabalho enriquece as evidências crescentes de que os HNBC não são mais seguros do que os TCC, sugerindo

que qualquer consumo de tabaco deve ser fortemente desincentivado.

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UMA TRIPLA
ASSOCIAÇÃO.
UMA ÚNICA TOMA³.
Um novo fôlego na DPOC.



LAMA | LABA | ICS



1 INALAÇÃO



1 VEZ AO DIA



1 DISPOSITIVO

ELEBRATO

FUROATO DE FLUTICASONA | UMECLIDÍNIO | VILANTEROL | ELLIPTA

ELE RESPIRA

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DPOC: Doença pulmonar obstrutiva crônica; ICS: Corticosteroide inalado; LABA: Agonista β2 de longa duração de ação; LAMA: Antagonista muscarínico de longa duração de ação.

INFORMAÇÕES ESSENCIAIS COMPATÍVEIS COM O RCM

▼ Este medicamento está sujeito a monitorização adicional. Isto irá permitir a rápida identificação de nova informação de segurança. Pede-se aos profissionais de saúde que notifiquem quaisquer suspeitas de reações adversas para <http://www.infarmed.pt/web/infarmed/submissaoram> e para o departamento médico da GlaxoSmithKline para lis.nucleo-farmacovigilancia@gsk.com.

NOME DO MEDICAMENTO Elebrato Ellipta **COMPOSIÇÃO QUALITATIVA E QUANTITATIVA** Cada inalação disponibiliza uma dose administrada de 92 microgramas de furoato de fluticasona, 65 microgramas de brometo de umecidínio equivalente a 55 microgramas de umecidínio e 22 microgramas de vilanterol (como trifenatato). Isto corresponde a um recipiente unidose de 100 microgramas de furoato de fluticasona, 74,2 microgramas de brometo de umecidínio equivalente a 62,5 microgramas de umecidínio e 25 microgramas de vilanterol (como trifenatato). Excipiente com efeito conhecido: cada dose administrada contém aproximadamente 25 mg de lactose mono-hidratada. **FORMA FARMACÉUTICA** Pó para inalação em recipiente unidose. **INDICAÇÕES TERAPÉUTICAS** Tratamento de manutenção em doentes adultos com doença pulmonar obstrutiva crónica (DPOC) moderada a grave, que não estejam adequadamente tratados com uma associação de um corticosteroide para inalação e um agonista beta-2 de longa duração de ação ou uma associação de um agonista beta-2 de longa duração de ação e um antagonista muscarínico de longa duração de ação (ver RCM para informação sobre efeitos sobre o controlo dos sintomas e prevenção das exacerbações). **POSOLOGIA E MODO DE ADMINISTRAÇÃO** Adultos A dose máxima recomendada é uma inalação 1x/dia, à mesma hora em cada dia. Doentes idosos, Compromisso renal e Compromisso hepático Não é necessário ajustar a posologia. Utilizar com precaução em doentes com compromisso hepático moderado a grave. População pediátrica A utilização não é relevante na população pediátrica (<18 anos) para a indicação de DPOC. Modo de administração Via inalatória. **CONTRAINDICAÇÕES** Hipersensibilidade às substâncias ativas ou a qualquer um dos excipientes. **EFEITOS INDESEJÁVEIS** As reações adversas mais frequentemente notificadas foram nasofaringite, cefaleia e infeção das vias respiratórias superiores. **Infeções e infestações** Frequentes Pneumonia, infeção das vias respiratórias superiores, bronquite, faringite, rinite, sinusite, gripe, nasofaringite, candidíase da boca e da garganta e infeção do trato urinário Pouco frequentes Infeção viral das vias respiratórias superiores **Doenças do sistema imunitário** Raras Reações de hipersensibilidade, incluindo anafilaxia, angioedema, urticária e erupção cutânea **Doenças do sistema nervoso** Frequentes Cefaleia **Afeções oculares** Desconhecido Visão turva **Cardiopatias** Pouco frequentes Taquiarritmia supraventricular, taquicardia e fibrilhação auricular **Doenças respiratórias, torácicas e do mediastino** Frequentes Tosse e dor orofaríngea Pouco frequentes Disfonia **Doenças gastrointestinais** Frequentes Obstipação Pouco frequentes Boca seca **Afeções musculoesqueléticas e dos tecidos conjuntivos** Frequentes Artralgia e dorsoalgia Pouco frequentes Fraturas **TITULAR DA AIM** GlaxoSmithKline Trading Services Limited, 12 Riverwalk, Citywest Business Campus, Dublin 24, Irlanda **DATA DA REVISÃO DO TEXTO** fevereiro 2021. **APRESENTAÇÃO:** Elebrato Ellipta 92 mcg+55 mcg+22mcg, 30 doses. **Regime de Participação:** Escalão B. Regime Geral 69%; Regime Especial 84%. 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. Medicamento sujeito a receita médica. 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 & Cª, S.A., -A Av. da Siderurgia Nacional, 4745-457 S.Mamede do Coronado; NIF: 500220913. **DMgM_PT210310**

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