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Sommaire

Torsion ovarienne chez un nourrisson de 1 mois : cas clinique et revue des possibilités thérapeutiques

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ABSTRACT

Ovarian torsion is a potential complication of antenatal ovarian cysts. These are the most common intra-abdominal cystic abnormality, affecting up to 1 in 1000 fetuses. Given the young age of patients, ovarian torsion due to antenatal cysts result in aspecific symptoms. Subsequently, ante- and post-natal follow-up facilitate rapid and appropriate management. We discuss the management of these cysts according to their size and ultrasound appearance. Currently, antenatal surgery is not supported due to the associated risks. Instead, a multidisciplinary surveillance approach is recommended, and monitoring of cysts should be regular after birth. In the majority of cases there is spontaneous resolution; however in the absence of regression or if symptoms appear, urgent surgery is recommended to preserve the child's fertility.

KEYWORDS

Fœtal ovarian torsion, ovarian cysts, pediatry, neonatal surgery.

Introduction

Une torsion ovarienne est un diagnostic différentiel auquel nous pensons chez les jeunes filles se plaignant de douleurs abdominales aigues mais beaucoup plus difficile d'approche chez les nourrissons en l'absence de diagnostic anténatal. Les kystes ovariens anténataux et néonataux sont des facteurs de risque très importants chez ces enfants qui présentent alors des plaintes aspécifiques. L'avenir de leur fertilité est pourtant en jeu d'où l'intérêt de dépister et suivre ces kystes suivant un protocole strict afin d'éviter les complications graves telles que la torsion ovarienne.

Cas clinique

Un nourrisson de 1 mois s'est présenté dans notre service d'urgence pour fièvre et altération de l'état général depuis le jour même. Un contexte d'inappétence est relevé par les parents depuis la veille. Il n'y avait pas d'antécédents personnel ou familial notables, pas d'anomalies retrouvées sur les échographies anténatales réalisées lors du suivi de grossesse.

A l'examen clinique, elle est grincheuse et marbrée sans foyer clinique évident. Les examens complémentaires relèvent une protéine C-réactive à 79mg/l, une procalcitonine à 0.39ng/ml, une hyperleucocytose à tendance neutrophile. Réalisation d'une ponction lombaire, d'une culture urinaire, de frottis viraux RSV et grippe et d'une hémoculture puis traitement empirique par amoxicilline/gentamycine. 48h post antibiothérapie, la culture urinaire revient positive pour un Gardnerella vaginalis et une échographie abdominale est réalisée dans ce cadre afin de rechercher une éventuelle pyélonéphrite. L'échographie retrouve un ovaire droit augmenté de volume et avasculaire laissant suspecter une torsion ovarienne droite.

Une chirurgie effectuée en urgence retrouve un ovaire droit nécrosé inflammatoire et surinfecté réalisant un plastron pelvien avec inclusion de plusieurs anses digestives et de l'appendice. Réalisation d'une salpingo ovariectomie et libération des anses digestives plus appendicectomie sous laparoscopie.

Discussion

Les kystes ovariens sont retrouvés lors des échographies anténatales chez 1 fœtus féminin sur 1000. Leur origine est à mettre en lien avec les hormones gonadotropes fœtales stimulées par les œstrogènes et la β-HCG maternels. Beaucoup d'entre eux vont régresser spontanément durant les premiers mois de vie, une fois cette ambiance hormonale passée. Notons l'augmentation de la fréquence de ces kystes lors de grossesses compliquées par un contexte de pré éclampsie, diabète, iso immunisation rhésus ou encore hypothyroïdie.

Un kyste est considéré comme physiologique s'il ne dépasse pas 20 mm de diamètre. Dans la majorité des cas, il s'agit de formations folliculaires mais très rarement, des cystadénomes ou tératomes ont été décrits dans la littérature.

Les kystes ovariens peuvent principalement se compliquer de rupture hémorragique ou de torsion ovarienne d'où la nécessité d'un suivi régulier.

De plus gros kystes peuvent aussi mener à un élargissement abdominal fœtal et rendre difficile la naissance par voie basse ou encore exercer une compression sur les systèmes digestif ou urinaire et mener à un poly hydramnios. Au-delà d'une certaine taille de kyste, le risque de lacération du parenchyme ovarien et de torsion de l'ovaire augmente de façon exponentielle.

L'attitude des obstétriciens face aux kystes compliqués en anténatale n'est pas encore bien définie. Les discussions portent sur la taille du kyste et sa morphologie qui peut être simple ou complexe à l'échographie. En fonction de ces caractéristiques, diverses attitudes peuvent être adoptées : une surveillance accrue sans intervention si le diamètre du kyste est inférieur à 4 cm et son aspect simple, une ponction du kyste anténatale ou post natale si diamètre supérieure à 4 cm et aspect simple et une prise en charge chirurgicale néonatale (laparoscopie avec kystectomie voire plus en fonction des lésions observées pouvant aller jusqu'à l'ovariectomie) ou une surveillance échographique (encore controversé à l'heure actuelle) si kyste d'aspect compliqué en post natal. La revue de différentes études a pour but ici de dresser quelques lignes de conduite.

Echographiquement, les kystes sont soit dits simples c'est-à-dire anéchogène, à parois fines et vides de tout débris ou compliqués c'est-à-dire avec des cloisons et des éléments internes pouvant signer une hémorragie ou une torsion. Un kyste simple peut devenir compliqué d'autant plus qu'il est volumineux (risque très majoré au-delà de 4 cm).

La démarche est, selon beaucoup d'articles, attentiste devant un kyste simple et de moins de 4 cm. Elle consiste en de nombreux contrôles échographiques, mensuels en anténatal et à J3, J21, M2, M4, M6, M12.

L'équipe du Dr Galinier ajoute un suivi biologique en dosant les hormones œstradiol, LH, FSH et les marqueurs tumoraux α-foeto protéine, antigène carcino-embryonnaire, β-HCG. Cette démarche n'est pas reprise par les autres publications mentionnées dans les références ci-dessous.

Au bout de 6 mois, la grosse majorité des kystes aura spontanément régressé mais si ce n'est pas le cas une chirurgie peut être proposée. De même si des plaintes surviennent ou si une croissance du kyste est observée.

Les parents doivent être formés à détecter les plaintes de leur enfant pouvant signifier une torsion du kyste.

Un article récent paru dans *Prenatal diagnosis* en mai 2020, retrace l'histoire naturelle de 102 kystes ovariens et a repris cette classification de kystes complexes versus simples et petits (<40mm) versus large (≥40mm). Le but est de renseigner leur évolution avec une attitude conservatrice dans le cadre d'un suivi échographique anténatal mensuel et directement après la naissance. L'incidence de résolution est significativement plus haute parmi les kystes simples par rapport aux kystes complexes, de même pour les petits kystes vs larges. Le risque de changement de pattern de simple vers complexe est plus fréquent dans le cas de kystes larges. L'article a mis en lien le risque de torsion plus important parmi les kyste complexe mais pas de façon significative pour les kystes larges seuls.

Un article londonien de 2017 soulève cependant que les kystes simples de plus de 40mm ont moins de chance de torsion ovarienne prénatale si une aspiration prénatale a eu lieu par rapport au groupe contrôle (surveillance uniquement). De même, les kystes aspirés ont moins de risques de nécessiter une chirurgie par après. Les kystes simples de diamètre compris entre 50 et 59 mm sont ceux les plus à risque et une aspiration peut donc être envisagée si le fœtus est bien positionné. Rappelons tout de même les complications rares mais pouvant survenir lors de l'aspiration d'un kyste in utéro : infection, déclanchement du travail prématuré, saignement. Une ré accumulation liquidienne peut aussi mener à une chirurgie par la suite.

Le taux de perte ovarienne dans les kystes compliqués varie de 44 à 89%. Ces chiffrent sont notamment expliqués par le fait que l'image échographique compliquée est mise en lien, au niveau chirurgical, avec des ovaires déjà tordus. Peu de complications telles qu'une surinfection, une hémorragie, la formation de brides intra abdominales ou l'omission d'une pathologie cancéreuse ont été décrites mais notre cas clinique prouve qu'une surveillance régulière doit être maintenue car bien que rares, les complications des kystes ovariens peuvent être graves.

Se pose ainsi la question de soumettre l'enfant aux risques liés à une anesthésie et chirurgie si l'ovaire est déjà perdu. Le consensus dégagé après plusieurs lectures est le suivant : si le kyste qui était simple lors des premières images, devient compliqué lors du suivi en post natal, une chirurgie est réalisée en urgence mais si la conversion a lieu en anténatal l'avenir du parenchyme ovarien est compromis sauf si le terme permet une chirurgie rapidement et dans les autres cas une ponction in utéro peut être proposée au-delà d'un diamètre de 40mm.

Qu'en est-il donc des fœtus avec un kyste qui se complique avant terme ? Faut-il provoquer la naissance pour sauver le parenchyme ovarien ? La sanction chirurgicale doit en tout cas être le fruit d'une discussion multidisciplinaire et en accord avec les parents.

Conclusion

Les kystes ovariens constituent les formations intra-abdominales les plus souvent retrouvées et concernent 1 fœtus sur 1000. Un suivi échographique attentif doit être réalisé en anténatal et en postnatal pour décider d'une éventuelle sanction chirurgicale. Les kystes simples dont le diamètre est inférieur à 40mm régressent pour la plupart dans les six mois qui suivent la naissance. Les kystes compliqués sont associés à un mauvais pronostic ovarien et traduisent fréquemment une torsion ovarienne.

La taille et l'apparence des kystes sont les déterminants majeurs de leur devenir périnatal, une prise en charge chirurgicale est encouragée en cas de kystes larges et complexes et proposée en cas de kyste complexes et petits. A ce jour il n'existe pas de consensus sur le bénéfice d'une intervention anténatale vu les risques associés de saignement, d'accouchement prématuré ou encore de perforation digestive. Les parents doivent être impliqués dans chaque étape décisionnelle et être en mesure de reconnaitre tout signe clinique pouvant suggérer une torsion.

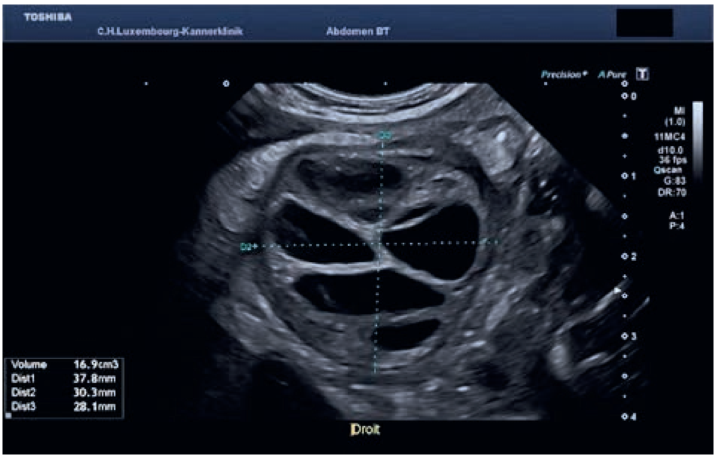

Figure 1: *ovaire droit composé de multiples follicules, augmenté de volume (4x3x3cm) et dans un environnement hyperéchogène et hypervasculaire.*

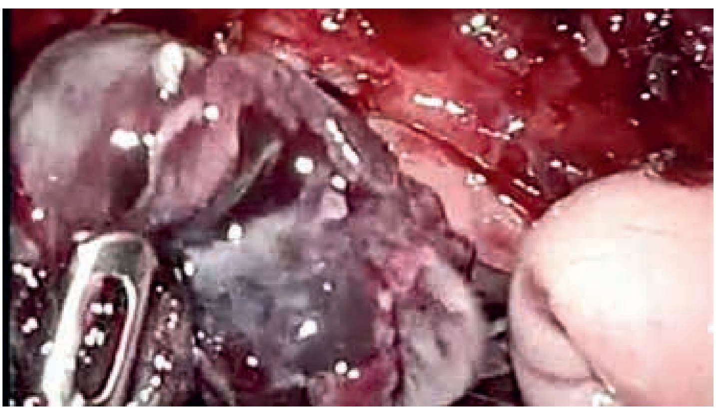

Figure 2 : *Ovaire droit nécrosé inflammatoire et surinfecté avec adhérence intestinale.*

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La péritonite infectieuse féline (PIF), une maladie complexe due à un coronavirus félin (FCoV)

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Le coronavirus félin appartient à la famille des Alpha-coronavirus[1] ; c'est un virus entérique très répandu dans les populations de chats, plus de 50% de la population féline sera en contact avec ce virus, ce chiffre peut monter jusqu'à 90% dans des environnements à forte densité de chats comme les élevages ou chatteries et qui provoque dans la vaste majorité des cas une simple entérite[2]. Le FCoV survit plusieurs semaines dans un environnement sec mais il est rapidement inactivé par les détergents et désinfectants. La transmission oro-fécale est classique pour un virus entérale.

Quand une entérite devient une maladie à médiation immunitaire

Cependant dans de rares cas, le virus passe des entérocytes dans les macrophages et les monocytes, où il va se répliquer activement et la maladie devient systémique. On parle alors de PIF (péritonite infectieuse féline) qui peut se présenter sous deux formes cliniques différentes (bien que souvent les deux formes soient présentes simultanément) : une forme exsudative (humide) caractérisée par une polysérite (péritonite, pleurite et péricardite) avec vascularite et une forme sèche se caractérisant par des lésions granulomateuses dans différents organes[5]. Il s'agit à ce stade d'une maladie à complexes immuns (antigène viral, anticorps, complément) qui attirent de plus en plus de macrophages et de neutrophiles avec formation de lésions pyogranulomateuses typiques sans pour autant la moindre activation des lymphocytes T cytotoxiques. Une fois la maladie déclarée, elle est pratiquement toujours mortelle[2].

La PIF est typiquement une maladie du jeune, voire très jeune chat (3 mois à 2 ans) avec une deuxième population qui développe la maladie plus tard (porteurs chroniques) [3]. Certaines races sont plus sensibles que d'autres pour développer la forme grave, entre autres le Persan et le Birman. La répartition géographique de la maladie est mondiale et affecte aussi les félins sauvages. Les guépards semblent particulièrement vulnérables[2].

Le passage du virus des entérocytes dans les macrophages et l'activation du tableau clinique de la FIP n'a longtemps pas été bien compris car dans une même populations la plupart des chats restaient en bonne santé et le développement de la maladie mortelle pour certains individus semblait être tout à fait aléatoire. Aujourd'hui on sait qu'il faut une mutation spontanée dans le génome du virus pour pouvoir envahir les macrophages et devenir le biotype viral pathogène (FIPV) responsable de la PIF. Entre 1.4% (population globale) et jusque 5% (chatteries) des chats infectés avec le FCoV vont développer la PIF[2, 4]. Ce biotype pathogène ne se reproduit presque plus dans les entérocytes. Deux hypothèses de transmission de la PIF sont discutées : la mutation spontanée du virus du biotype entérale dans le chat infecté avec passage dans les macrophages et cloisonnement dans le chat sans excrétion et sans transmission à d'autres chats et l'hypothèse de la circulation de virus du biotype viral pathogène entre chats. La plupart de chercheurs privilégient aujourd'hui la première hypothèse.

Signes cliniques de la PIF

Etant donné que le virus de la PIF peut affecter tous les organes, les symptômes sont variés et non spécifiques :

- Fièvre et apathie
- Anorexie
- Perte de poids
- Iridocyclite
- Signes neurologiques (environ 10% des cas)
- Polysérite et épanchements dans la forme humide (la forme la plus fréquente)

Diagnostic

- Une prise de sang révélera souvent des anomalies hématologiques (anémie non-régénérative, lymphopénie, neutrophilie et thrombocytopénie) et biochimiques (hyperprotéinémie et surtout hyperglobulinémie avec hypoalbuminémie et hyperbilirubinémie) sans être diagnostique.
- Une sérologie avec recherche d'anticorps FCoV est d'une utilité restreinte car plus de 50 % des chats auront été en contact avec le biotype entérale et donc séropositifs et certains chats qui ont la PIF auront une sérologie négative car tous les anticorps peuvent être liés à des complexes immuns.
- En cas de forme humide l'analyse du liquide d'épanchement est très important.
    - Le test de Rivalta doit être fortement positif ; un test négatif peut exclure la PIF, un test positif n'est pas diagnostique.
    - RT-PCR sur épanchement : haute spécificité (83-98%) mais sensibilité de 40 à 83 % selon les études[8].
- Analyse du liquide cérébro-spinal et de l'humeur aqueuse.
- Pour les formes sèches et les formes humides avec tests non concluant il

faudra recourir à des biopsies de tissus obtenues par ponction échoguidée, laparoscopie ou laparotomie ; l'histologie est pathognomonique et il est possible de mettre en évidence des cellules exprimant les antigènes FCoV par IF ou immunohistochimie sur les pyogranulomes dans des laboratoires spécialisés (golden standard).

- Recherche des FCoV qui présentent la mutation de la forme pathogène. Plusieurs mutations pour la forme pathogène ont été décrites (sur des protéines accessoires et surtout pour la protéine Spike) mais aucune validation est disponible aujourd'hui, il semble en plus qu'il y ait de larges différences géographiques[4, 6].

Le diagnostic de la PIF reste aujourd'hui difficile mais devient de plus en plus important au vu de l'émergence d'un nouveau traitement[2, 4, 5, 8].

Prévention

Il existe un vaccin intranasal contre la PIF (virus modifié sensible à la température qui ne se réplique que dans le système respiratoire supérieur), il n'est pas considéré comme 'core vaccine' par les spécialistes en médecine féline du 'European Advisory Board on Cat Diseases'. En effet il n'est d'aucune utilité pour les chats ayant déjà été en contact avec le FCoV, il peut cependant jouer un rôle dans des élevages pour les chats séronégatifs à introduire dans une population porteuse du virus[6, 7].

Traitement

Aucun traitement efficace contre la PIF était connu avant 2018 quand Pedersen et son équipe de l'université de Davis, Californie, publiaient des résultats encourageants obtenus en utilisant les analogues de nucléosides GS-441524 et GS-5734 de Gilead Sciences Inc. (California), le dernier mieux connu aujourd'hui sous le nom de Remdesivir[9]. Si les essais préliminaires in vitro et en laboratoire étaient bien concluants et ressemblaient à un véritable miracle, ces analogues de nucléosides étaient testés au même moment cliniquement sur le virus Ebola et Gilead Sciences Inc. voulait réserver ces molécules à la médecine humaine. Un certain coronavirus humain SARS-CoV2 viendra perturber et retarder de nouveau une éventuelle mise sur le marché pharmaceutique vétérinaire de ces molécules en 2020, ceci après des essais cliniques sur des chats malades de la FIP et pour la première fois guéris, certains endéans quelques jours par ce nouveau médicament (bien qu'il y ait eu quelques rechutes)[10].

L'histoire miraculeuse de la nouvelle molécule qui peut guérir une maladie jusque-là mortelle deviendra ensuite kafkaïenne[11].

On effet une firme chinoise voulait produire la molécule, mais elle n'obtint pas la licence de Gilead Sciences Inc. Peu de temps après la molécule était cependant disponible en Chine sur le marché noir et pouvait être commandée par des particuliers sur internet moyennant payement de plusieurs milliers de dollars.

Etant donné que la maladie est répartie mondialement et surtout dans des chats de races, une communauté de propriétaires nord-américains et européens s'organisait sur les réseaux sociaux pour commander cette molécule miracle en Chine et sauver ainsi leurs chats atteints de PIF d'une mort certaine. Encore fallait-il trouver des vétérinaires coopérants car il faut injecter la 'molécule chinoise' en sous-cutané toutes les 24 heures, acte très douloureux difficile à réaliser par les propriétaires. Si on pouvait parler d'un certain dilemme éthique pour les vétérinaires (laisser mourir ou sauver l'animal en utilisant une molécule d'origine inconnue, envoyée dans des emballages vierges, ne respectant pas la propriété intellectuelle), au moins la situation juridique était claire et la grande majorité de la communauté professionnelle refusa l'administration du traitement ; cependant la molécule chinoise a été utilisée des milliers de fois à travers le monde, aussi au Grand-duché. Évidemment apparurent bientôt des lots vendus par des mafieux de tout genre (prix exorbitant pour une petite quantité de liquide livré vierge de toute note ou numéro de lot) qui non seulement ne guérissaient pas les chats mais pour certains lots les tuaient.

Ce n'est que fin 2021 que finalement trois médicaments à base de Remdesivir sont disponibles pour usage vétérinaire, pour le moment uniquement en Grande-Bretagne (malheureusement hors UE) et en Australie[12]. Par contre le traitement reste très cher car long et les recherches sur d'autres molécules continuent (p. ex. la méfloquine, traitement anti-malaria en médecine humaine montre une inhibition du FIPV in vitro).

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Exploring the association between patient characteristics and emergency department use in Luxembourg

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KEYWORDS

Emergency Department, General Practitioner, Luxembourg, Quality and Costs of Primary Care (QUALICOPC), multi-level logistic model, patient characteristics

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Abstract

Background

In recent years, many countries including Luxembourg have faced the challenge of high use of Emergency Departments (EDs). High ED use can create inefficiencies in service delivery and result in poor patient outcomes. In order to address the problem of high demand for ED care, it is necessary to identify the characteristics of patients who use EDs. The aim of this study is to explore the relationship between patient characteristics and ED use in Luxembourg.

Methods

We used data for Luxembourg collected as part of a European study on the Quality and Costs of Primary Care (QUALICOPC) between October 2011 and December 2013. Survey data was collected from 713 adult patients, aged 18 years and over who attended 80 primary care practices. The dependent variable was a binary variable on ED use equal to one if a patient visited an ED at least once during the previous 12 months. Multilevel logistic regression analysis was used to identify patient characteristics associated with ED attendance.

Results

Overall, 34% of participants attended the ED at least once during the previous 12 months. Patients aged 50-65 years (OR 0.38, 95% CI 0.22 – 0.67) or 65 years and over (OR 0.27, 95% CI 0.12 – 0.64) were less likely to attend the ED. Patients with poor self-reported general health were more likely to visit the ED compared to patients who reported very good health (OR 3.48, 95% CI 1.52 – 7.99).

Conclusions

Since the survey was undertaken, the government has introduced several policies to address the high use of EDs. Future research could collect new data in order to assess these policies and also investigate supply-side factors.

Introduction

Emergency departments (EDs) play an important role in the health system by providing rapid access to care for urgent medical needs (1, 2). From a patient's perspective, the choice to seek care in an ED depends on both individual characteristics and contextual factors including deprivation, access to primary care and continuity of care (1, 3, 4).

In recent years, the use of EDs has increased in many countries (5). Increased pressure on EDs can affect the provision of high quality care and result in overcrowding, treatment delays, reduced patient satisfaction and clinical outcomes and inefficient services (1, 4, 6). In order to address the issue of high ED use, policymakers often focus on three groups: 1) inappropriate users, 2) older people, in particular those aged 85 years and over and 3) frequent users (4). Inappropriate use of EDs is typically defined in relation to low urgency, self-referred patients who do not require admission and who could be better managed by other services (1). Inappropriate visits to EDs are estimated to vary between 20% and 40% depending on the country and the definition used to define appropriateness (1, 6, 7). Potential factors associated with the inappropriate use of EDs include not having a regular primary care provider and problems accessing primary care (8-10). High ED use by older people is primarily driven by higher need arising from multimorbidity and frailty, which can be exacerbated by a lack of material or social support (4). Frequent ED users are often frequent users of other health and social care services and require coordinated and continuing care to meet their complex needs, which cannot be adequately addressed by ED services alone (2, 4).

In Luxembourg, emergency services are provided by each of the four general hospitals. Out-of-hours care primary care is provided by three medical on-call centres *(maisons médicales de garde)* located in the centre, north and south of the country (11). The *maisons médicales de garde* were created in 2008 but for several years following their creation, many patients did not know how to access these services and there was poor coordination between the *maisons médicales de garde* and EDs. Therefore, the *maisons médicales de garde* were viewed as unsuccessful in diverting care from EDs (12, 13). In 2017, the Ministries of Health and Social Security published a study investigating the functioning of emergency services in Luxembourg (14). This brought attention to several issues including high demand for emergency services leading to waiting times for patients. Moreover, a notable percentage (18.5%) of the activity of emergency services related to visits for low-severity issues (15).

This paper investigates patient characteristics associated with the use of EDs in Luxembourg. The results can help to identify solutions to address high use of EDs in Luxembourg.

Methodology

We used data from the Quality and Costs of Primary Care (QUALICOPC) project, an international study in 34 countries coordinated by NIVEL (the Netherlands Institute for Health Services Research) and co-funded by the European Commission. The main objective of this study was to evaluate primary health care in terms of quality, equity, and cost. Full details of the QUALICOPC study methodology is available as a published article (16).

Data collection

Data collection took place between October 2011 and December 2013. A nationally representative sample of GPs and patients completed validated questionnaires. The study included GPs as the main primary care providers with one GP per practice eligible for participation in order to avoid having multiple GPs from the same practice who were not independent. GPs who were retired, no longer practicing, or practicing in another country were excluded from the study (16). Participation was voluntary and in Luxembourg, almost all GPs (120) were invited via telephone to participate in the study, of which 78 agreed (response rate of 65%). The reasons for GP non-participation were not recorded. The participating GPs filled in a questionnaire which consisted of questions on the context of the practice, the human resources and equipment, the employment status of the physician and the structure of the practice, the usual care processes, and the physician activity profiles (16).

The study adopted a convenience sampling approach for patients. Trained fieldworkers recruited ten patients who had just consulted each participating GP in the practice waiting room. Participation was voluntary with patients invited to complete a questionnaire. In each practice, nine patients completed a questionnaire about their experiences of the consultation they just completed, while one patient completed a questionnaire about what they valued and rated most important about the completed consultation (16). These proportions of patients were based on the findings of previous studies of large variation in patient experiences and smaller variation in patient values within a country (16). The patient experience questionnaire contained questions on the use of EDs. 792 patients aged 18 years and over participated in the study with a participation rate of 72%. Of these, 713 patients completed the patient experience questionnaire and 79 patients completed the patient values questionnaire. Reasons for non-participation were not recorded. Patients who visited the GP only for administrative tasks (for example to collect a medical certificate) or who were too unwell to answer the questions were excluded.

GPs and patients in the study provided informed consent to participate. The study was conducted following the guidelines of the National Committee of Research Ethics (CNER).

Outcome variable

The outcome variable was self-reported use of EDs in the previous 12 months in response to the question: “in the last 12 months, how often did you visit a hospital emergency department for yourself?”. Answers included: “never, one time, two or three times, four or more times”. We created a binary variable equalling zero if patients never visited an ED in the previous 12 months and equalling one if patients made at least one visit to an ED in the previous 12 months.

Independent explanatory variables

Independent variables were socio-demographic and health characteristics of patients including age, gender, education, employment, ethnicity and health status. Age was categorized into four groups: 18–33 years, 34-49 years, 50–65 years, and over 65 years. We included patients' socio-economic status using variables on education and employment. Education was evaluated using three categories: 1) educated up to lower secondary level, 2) upper secondary education only, and 3) post-secondary education. Employment was evaluated using four categories: 1) employed (including self-employed), 2) unemployed, 3) retired and 4) unavailable for work (including students, homemakers and those unable to work due to illness or disability). Two variables captured immigration status "mother birthplace" and "patient birthplace". When both the mother and patient were born in the country of residence or when only the mother was born in the country of residence, we considered the patient a non-immigrant. When both patient and mother were born outside the country of residence, we considered the patient as a "first generation migrant". When the patient was born in the country of residence and the mother was born in a foreign country, we considered the patient a "second generation migrant" (17). Self-reported health status was evaluated using two variables: 1) the presence of a longstanding illness or condition (no or yes) and 2) self-assessed general health status divided into four categories (very good, good, fair and poor).

Data Analysis

We used multilevel logistic regression to analyse patient characteristics associated with ED use. The use of a multilevel model allowed us to model the cluster structure of our data (patients nested in GP practices) and to consider the heterogeneity between practices (18, 19). First, we estimated a null (or empty) model, which included the dependent variable (ED visit in previous 12 months) and a random effect at the practice level and did not include any independent explanatory variables. The aim of this first step was to estimate intergroup heterogeneity using the intraclass correlation coefficient (ICC), which quantifies the amount of variation in the outcome variable explained by the practice level. Second, we estimated a multilevel multivariate logistic regression model, adjusted for patient characteristics. We report results as adjusted Odd Ratios. All statistical analyses were conducted using Stata version 15 (20).

Results

Descriptive statistics

In our estimation sample, the prevalence of ED attendance in the previous 12 months was 34%. Table 1 displays the characteristics of patients who did and did not attend an ED in the previous 12 months. A higher percentage of patients who were aged 18-33 years reported an ED visit in the previous 12 months. A higher percentage of second-generation immigrants reported an ED visit. An ED visit was more common among patients educated up to lower secondary level. An ED visit was also more prevalent among unemployed patients. A higher percentage of patients with poor health or a long-standing illness or condition also reported an ED visit in the previous 12 months.

Table 1. *Patient characteristics by ED visit, n=615*

ED visit				
	No		Yes	
Patient characteristics	**N**	**%**	**N**	**%**
Sex				
Male (reference)	179	66	93	34
Female	227	66	116	34
Age				
18-33 (reference)	76	57	57	43
34-49	127	62	79	38
50-65	125	72	49	28
65 and over	78	76	24	24
Immigration status				
Non-immigrant	192	71	80	29
First generation immigrant	171	65	94	35
Second generation immigrant	43	55	35	45
Education				
Up to l	83	62	50	38
Upper secondary only	192	66	101	34
Post-secondary	131	6	58	31
Employment				
Employed	230	65	126	35
Unemployed	8	50	8	50
Retired	113	74	40	26
Unavailable for work	55	61	35	39
Self-assessed general health				
Very good (reference)	81	77	24	23
Good	195	68	93	32

Fair	107	60	71	40
Poor	23	52	21	48
Long-standing illness or condition				
No (reference)	274	68	128	32
Yes	132	62	81	38

Multilevel logistic regression models results

The intraclass correlation coefficient (ICC) from the null model (with no patient characteristics included) was 0.01, which indicated that only 1% of the total variance of ED use was explained by the practice level. Therefore, most of the variation in patients' ED use was at the patient level and can therefore be assigned to patient characteristics.

Table 2 displays the results of the multilevel logistic regression. Compared to patients aged 18-33 years, patients aged 50-65 (OR 0.38, 95% CI 0.22 – 0.67) and patients aged 65 years and over (OR 0.27, 95% CI 0.12 – 0.64) were less likely to use the ED in the previous 12 months. Compared to patients who reported very good general health, patients who reported good (OR 1.86, 95% CI 1.08 – 3.19), fair (OR 2.41, 95% CI 1.32 – 4.38) or poor (OR 3.48, 95% CI 1.52 – 7.99) health were more likely to report an ED attendance in the previous 12 months.

Table 2. *Results of multilevel logistic regression, n=615*

Variable	Odds Ratio	95% confidence interval	p-value
Sex			
Male			
Female	0.89	0.62 – 1.28	0.530
Age			
18-33			
34-49	0.78	0.49 – 1.25	0.308
50-65	0.38	0.22 – 0.67	**0.001**
65 and over	0.27	0.12 – 0.64	**0.003**
Immigration status			
Non-immigrant			
First generation immigrant	1.08	0.73 – 1.59	0.689
Second generation immigrant	1.54	0.89 – 2.68	0.125
Education			

Up to lower secondary level			
Upper secondary only	0.78	0.49 – 1.23	0.284
Post-secondary	0.62	0.37 – 1.05	**0.073**
Employment			
Unemployed	1.84	0.65 – 5.26	**0.253**
Retired	1.07	0.55 – 2.06	**0.845**
Unavailable for work	1.05	0.62 – 1.75	**0.866**
Self-assessed general health			
Very good			
Good	1.86	1.08 – 3.19	**0.024**
Fair	2.41	1.32 – 4.38	**0.004**
Poor	3.48	1.52 – 7.99	**0.003**
Long-standing illness or condition			
No			
Yes	1.37	0.92 – 2.05	0.121
Constant	0.50	0.24 – 1.06	0.072

Discussion

Overview of study findings and comparison with previous literature

ED overcrowding is a long-standing international concern and places a burden on health systems (21). This cross-sectional study provides an overview of primary care characteristics associated with ED attendance in Luxembourg. We found that patients attending EDs were significantly more likely to have poor or very poor self-reported health. Older patients were significantly less likely to attend the ED compared to younger patients. These findings reflect those of a study that examined use of EDs using data for all countries that participated in the QUALICOPC project (6).

The inverse relationship between ED visits and age has also been identified elsewhere (7, 22). This may indicate lack of understanding regarding the appropriate use of ED services or knowledge of alternative community services among younger people who may have less contact with the health system. Moreover, it is important to distinguish between appropriate and inappropriate visits. Younger people may be more likely to have inappropriate visits while visits by older people may be more likely to be appropriate as they have a higher prevalence of chronic conditions and may receive complementary tests and medication and a large proportion of older people require hospital care upon presentation at the ED (4, 7). In our study the inverse relation between ED and age was significant after adjustment for the presence of a chronic illness or condition. Our findings suggest that targeted education to young adults about ED use may help to address ED overcrowding.

Study limitations and future research

This study has several limitations. First, there are limitations inherent to survey and secondary data, including missing data or selection bias (for example, if patients were excluded because they did not speak the same language as the interviewers). Due to the large amount of missing values, we were unable to analyse the reasons for choosing the ED as the point of care, which would have allowed us to obtain some insight into patients' perspectives for seeking care in the ED versus in out-of-hours primary care. Second, our survey data does not enable us to distinguish between appropriate and inappropriate users of EDs. In addressing ED overcrowding, ideally we would like to focus on inappropriate users and future research could attempt to identify inappropriate users, for example patients who were not admitted to hospital following the ED visit. Third, due to the small sample size, we did not investigate the association between patient characteristics and ED use for frequent ED users. Fourth, our study is restricted to patients who visited a general practice and may not be representative of the general population. Furthermore, our sample does not include paediatric patients, who have been shown in the literature to be high users of ED services (1, 23). Despite these limitations, which could potentially be overcome in future data collection and research, this study presents a first attempt to understand the characteristics of individuals in contact with primary care who used EDs in Luxembourg.

Since the QUALICOPC study was undertaken, the government has introduced several measures to reduce demand for EDs including a financial incentive to support the development of primary care group practices offering extended opening hours (24), a public information campaign on care options for different severities of conditions (14) and a free mobile app allowing users to find medical practices in their locality (including outside of usual opening hours) (25). The government also provided funding to recruit extra staff for EDs and to undertake renovations to improve patient flow and prioritization (14). Future research could update this analysis and assess the effect of these policies. As we have focused on the demand for EDs, future research could also investigate supply-side factors including availability of services and staff. Emergency care plays an important role in the health system and it is essential that efficient and high-quality care is delivered to ensure patients' outcomes are maximised.

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Analysis of 30 years of pollen data in Luxembourg, characteristics and trends

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Abstract

BACKGROUND

For the past 30 years, the team of the aerobiological station at the Centre Hospitalier de Luxembourg (CHL) has been monitoring atmospheric pollen concentrations, to provide timely information to allergic patients about the presence of allergenic pollen in the air. An impressive amount of pollen data has accumulated, covering daily measurements for 30 seasons of 33 plant species.

OBJECTIVE

To characterise the data of 6 major allergenic pollen types and identify long-term trends in pollen concentrations and the timing of pollen seasons.

METHODS

pollen concentrations, peak days, season start, end and duration of the yearly pollen seasons were analyzed for *Corylus, Alnus, Betula, Quercus, Poaceae* and *Artemisia*. Long Term trends were tested for significance and clinical implications.

RESULTS

For the early flowering trees, *Corylus* and *Alnus* which pollinate in winter, there is a significant trend for an earlier start, a longer duration and a higher amount of seasonal pollen. For spring-pollinating trees *Betula* and Quercus, a trend for an earlier start and for higher pollen concentrations is observed. For the herbaceous plants, *Poaceae* and *Artemisia* pollinating in summer, a very significant decrease in pollen concentrations combined with an earlier season start for *Poaceae* was seen. Conclusion: An earlier start of most of the pollen seasons combined with a significant increase in pollen concentration for most allergenic tree pollen, in contrast to a significant decrease in herbaceous pollen concentrations, impacts on the clinical symptoms of pollen allergic patients.

KEYWORDS

Pollen, phenology, allergy, trends, climate, Luxembourg

ABBREVIATIONS

APIn: annual pollen integral ; CI: 95% confidence interval ; IQR: interquartile range; LR: linear regression ; MK: Mann-Kendall trend test ; TS: Theil-Sen estimator ; EAN: European Aeroallergen network; EAACI: European Academy of Allergy and Clinical Immunology.

SYNONYMS

Corylus = hazel (= Hasel, noisetier, Hieselter)
Alnus = alder (= Erle, aulne, Alënter)
Betula = birch (=Birke, bouleau, Bierk)
Quercus = oak (= Eiche, chêne, Eech)
Poaceae = grasses (Gräser, graminées, Grieser)
Artemisia = mugwort (Beifuss, armoise, Alzem)

Introduction

Airborne pollen and, to a lesser extent, mould spores are the cause of seasonal inhalant allergy symptoms in a major proportion of atopic persons. Rhinitis and asthma caused by a sensitization to pollen allergens affect between 20-30% of persons in north-western Europe (1,2). Conjunctivitis, rhinitis and asthma are triggered when a threshold of the atmospheric pollen which a person is sensitized to, is reached (3,4). This threshold is affected by the pollen type, with pollen allergenicity varying between different tree and herbaceous species. The pollination seasons occur in successive, sometimes overlapping waves, with tree pollen dominating winter and spring, and herbaceous pollen dominating late spring and summer. The start, end and intensity of these pollination seasons vary considerably from year to year and are dependent on weather conditions including temperature, rainfall, and insolation. To inform allergic patients about the outdoor concentration of allergenic pollen, reliably and on a day-to-day basis, the Luxembourg Ministry of Health together with the Health Directorate, and the Centre Hospitalier together with its

Unit of Immunology jointly set up an aerobiological unit for medical purposes. The “Pollen Station" has been operational since 1991. Information has been provided on a daily basis, initially via an automated phone reply system and regular radio and TV communications. Meteorological data and mean daily temperatures were communicated by the direction of the meteorological station of the airport of Luxembourg. Since 2005, pollen and mould spore concentrations could be accessed from the internet site at www.pollen.lu, which makes both current and historic pollen and mould spore concentrations publicly accessible. In 2002, the aerobiological unit became a member of the European Aeroallergen network (EAN).

Over the last few years, reports on the long-term trends in pollen season characteristics and climate change have been published by pioneers in pollen monitoring, covering sufficiently extended periods of pollen measurements (5–7). On the occasion of 30 years of pollen recording in Luxembourg and as a follow-up publication of a recent paper (8), this article is addressed more specifically to readers interested in the medical impact of pollen trends in Luxembourg. Previously unpublished data including the pollen seasons of *Alnus* and *Corylus* during the 1990-2000 decade are included.

Methods

Pollen sampler and location

The sampler, a Hirst-type 7-day volumetric spore trap (9) (Burkard Manufacturing CO, LTD, UK) has been located for the whole recording period on the roof of the Centre Hospitalier de Luxembourg, approximately 20 m above ground. The CHL is situated at an altitude of 322 m above sea level, and at latitude and longitude coordinates of 49° 37’6.2436 ‘’ N, 6°6’2.34 ‘’ E, on the western outskirts of the city of Luxembourg, which itself is situated in the southern part of the Gutland, a large plateau of early Jurassic sandstone formation. In and around Luxembourg city, the Alzette river and its affluent brooks have carved deep valleys into the sandstone plateau.

Pollen was analysed according to the guidelines of the European Aerobiology Society (10). Average daily pollen concentrations given as pollen /m^3 , were calculated after counting 2 longitudinal lines of daily slides. Sampling, manual identification and counting of pollen by microscope has been carried out by the same core team over all the years. Recommended terminology for aerobiological studies , as defined recently, was used throughout the paper (11).

Pollen species selected and vegetation

Of the 33 pollen taxa regularly recorded, we selected for the purpose of this paper, 4 tree pollen types (*Alnus*, *Corylus*, *Betula*, and Quercus) and 2 herbaceous pollen types

(*Poaceae* and *Artemisia*). These pollen types have the greatest allergy potency and their specific flowering periods occur sequentially over the first 8 months of the year. The sampling period of each year started in January and ended in September, except for 1991 when the pollen trap was only operational from 1. of March onwards. As a result of technical faults, the pollen sampler was not functional in 1992 from 2.8-13.8 in 1994 from 27.8-20.9, and in 2003 from 5.2 -19.2. Therefore, the years 1991 and 2003 were omitted for *Alnus* and *Corylus* evaluation of the season start and duration. Two short missing periods early and late August of the 1992 and 1994 seasons had no significant impact on *Artemisia* season characteristics. Interestingly, given the corresponding pollen season intensity, 28% of the surface of Luxembourg is occupied by meadows or pasture, 25 % is covered by deciduous trees (12). Of these, 28% are oak trees, 1,6% birch trees, while hazel and alder represent less than 2,1% of deciduous trees (13).

Season definitions

The total taxon-specific airborne pollen counted during a year is expressed as the Annual Pollen Integral (APIn) (8,9,11). For 2 years, we included *Corylus* and *Alnus* pollen observed in December in the APIn. The peak value is the highest daily concentration for a specific pollen, and the peak day is the day on which this maximum is observed.

There are different ways of defining the start and end dates of the pollen season of a specific taxon. We used retrospective percentile definitions, and prospective threshold definitions, as indicated in the Table below. The length of the season is obtained by subtracting the start day from the end day.

For birch pollen and grass pollen, we used two prospective threshold methods of interest for clinical studies. For birch, day one of the season is the 1st day of 5 days with ≥ 10 pollen /m^3 (out of 7 consecutive days) and when the sum of 5 days is ≥ 100 pollen /m^3. The end of the season is the last day of 5 days with ≥ 10 pollen /m^3 (out of 7 consecutive days) and the sum of 5 days is ≥ 100 pollen/m^3. For *Poaceae* the start of the season is the 1st day of 5 days with ≥ 3 pollen/m^3 (out of 7 consecutive days) and where the sum of 5 days is ≥ 30 pollen/m^3. The end of the season is the last day of 5 days with ≥3 pollen/ m^3 (out of 7 consecutive days) and where the sum of 5 days is ≥30 pollen/m^3. (14).

Name	Start	End	type
perc100	day of first pollen grain	day of last pollen grain	percentile
perc98	day of 1% of APIn	day of 99% of APIn	percentile
perc90	day of 5% of APIn	day of 95% of APIn	percentile
EAN	1% of APIn	95% of APIn	percentile
tr20	first day ≥20/m^3	last day ≥20/m^3	threshold

tr30	first day ≥30/m³	last day ≥30/m³	threshold
moving	first of 5 consecutive days with moving average ≥5/m³	first of 5 consecutive days with moving average ≥5/m³	threshold
EAACI	birch: first of 5 days with ≥10/m³ (out of 7 consecutive days), and a sum of 5 days ≥100/m³	birch: last of 5 days with ≥10/m³ (out of 7 consecutive days), and a sum of 5 days ≥100/m³	threshold

Pollen concentration levels of clinical relevance

For the evaluation of the clinically relevant exposure to pollen concentrations we used a slightly modified version of the Table first presented by W. Kersten and P.G. Von Wahl (4) and in use by the German Meteorological service (3).

	No Exposure	Weak Exposure	Medium Exposure	High Exposure
Tree pollen	0	1-10	11-50	> 50
Grass pollen	0	1-5	6-30	> 30
Artemisia pollen	0	1-2	3-6	>6

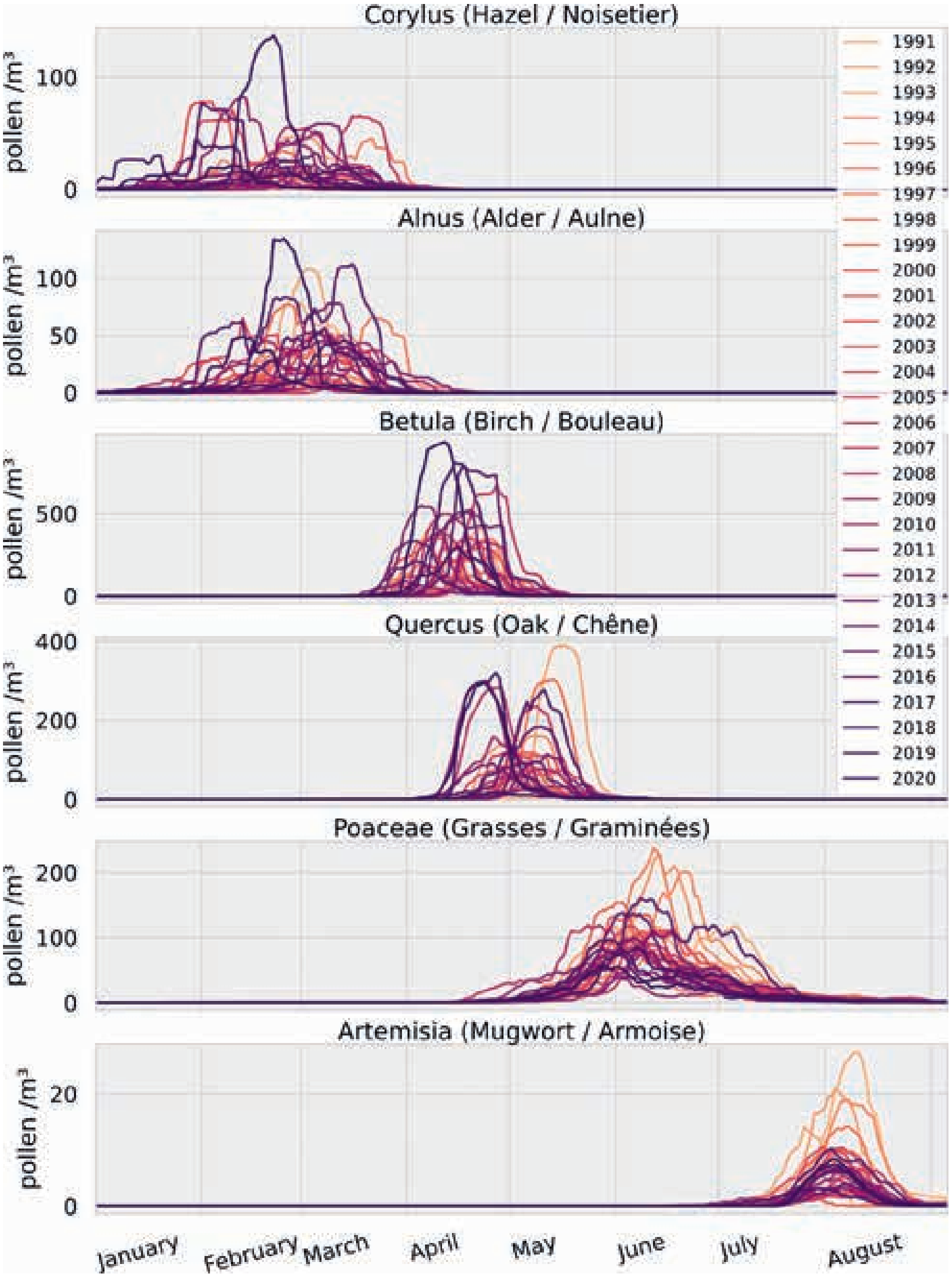
Pollen concentrations are given as daily concentration in pollen grains/m^3.

Data analysis

All data analysis was carried out in the Python environment (19) using a Jupyter notebook. Calculations used the Python libraries Pandas, Numpy, Scipy, Statsmodels, while data visualisation used matplotlib and Seaborn (box plots, stripplot) (20–25). For measures of pollen concentration (APIn, season peak value, season daily median), the average was calculated as the median due to the right-skewed distribution and the presence of outliers. 95% confidence intervals for the median were determined using bootstrapping with 20000 iterations.

For trend analysis of pollen season characteristics, we used linear regression (statsmodels) when the assumptions of the ordinary least squares method were met due to the higher statistical power of linear regression compared to non-parametric methods. Residuals tested for normality using the Shapiro-Wilk test, for homoscedasticity using the Breusch–Pagan test, and for absence of autocorrelation using the Ljung–Box test. Some measures required logarithmic transformation to reveal a trend that could be modeled using linear regression. For the pollen season characteristics that did not meet the assumptions of linear regression, the presence of a trend was tested using the Mann-Kendall hypothesis test (pyMannKendall library (26)), and the magnitude of the trend was estimated using the Theil-Sen estimator.

Trends in the mean temperature and precipitation were determined using data for the years 1990-2020 of the Copernicus ERA5 dataset (27) for the location of the pollen sampler. To estimate the trend in mean daily temperature for each day of



the year, we carried out a seasonal Kendall test (pyMannKendall library), in a rolling manner for each day of the year, each time using the period covering the 15 days before and the 14 days after the day in question. For each day, the slope of the seasonal Kendall test was used as the measure of the change from 1990 to 2020. To study trends in precipitation likely to affect pollen concentrations, we determined the total precipitation in the 14 days preceding a day. Then a seasonal Kendall test was carried out in the same manner as for the mean temperature.

Results

A. Allergenic pollen is released in distinctive but highly variable seasons

The pollen counts that have been carried out daily represent a wealth of data accumulated over 3 decades. To gain an overview of the complex phenomenon of pollination, we started by visualising the observations of the 6 main allergenic pollen species. We plotted 30 successive pollen seasons from the years 1991 to 2020, using the 14-day rolling average of daily pollen concentrations (Fig 1). Each plant pollinates at a specific time of the year: *Corylus* and *Alnus* produce pollen in late winter and early spring. This is followed in spring by *Betula*, then by Quercus. *Poaceae* pollen is produced in late spring and summer, while *Artemisia* produces pollen in the second half of the summer.

Seasonal pattern of the 6 pollen species under study over 30 years

FIG 1. *30 pollen seasons of the 6 main allergenic pollen species The 14-day rolling mean of daily pollen concentrations/m3 is shown for 1991-2020. For each year a different colour is used.*

The general aspect of a pollen season resembles a bell curve: a slow start, an intense period around the peak in the middle, and a slow decline at the end of the season. Using a 14-day rolling mean of daily concentrations smoothes over day-to-day variability resulting from weather changes.

While the sequence of pollen seasons is characteristic for each plant and repeated annually, there is large variability at several levels: Within each plant species, there is considerable variability between different years in terms of the amount of pollen produced and the season timing ; within each season, there is also variability in the amount of pollen produced each day. Finally there is variability between different plants in terms of the amount of pollen a plant characteristically produces.

To capture this variability and describe the pollen seasons we used different season characteristics, shown in Table I. These characteristics quantify aspects such as the amount of pollen produced in a season, the start of the season as well as its duration. From the perspective of the allergy sufferer, the severity of symptoms is related to the number of pollen grains they are exposed to. Another important parameter is therefore the period of time during which pollen concentrations are above subclinical levels.

TABLE I. *Key characteristics of pollen seasons, 1991-2020 in Luxembourg*

	APIn (pollen/m³)	Peak value	Typical day (pollen/m³)	Season start (perc90)	Season end (perc90)	Duration (perc90)
Corylus	**568** (619-1015) range:213-2271	**112** (127-214) range:22-473	**4** (4.2-6.2) range:2-16	**3.Feb** (30. Jan-10.Feb) 10.Jan-10.Mar	**15.Mar** (10. Mar -16.Mar) range: 26.Feb-29.Mar	**38.5 days** (33-42) range:11-67
Alnus	**912** (901-1368) range:252-3929	**158** (149-223) range:39-479	**6** (5.3-7.4) range:1-14	**15.Feb** (9.Feb -19.Feb) 21.Jan-14.Mar	**16.Mar** (15. Mar -21.Mar) range: 2.Mar-7. Apr	**34.0 days** (28-37) range:11-56
Betula	**5532** (5162-7297) range:1887-14134	**1209** (1102-1597) range:304-3200	**12** (12-18) range:4-39	**8.Apr** (6.Apr -11.Apr) 27.Mar-22.Apr	**27.Apr** (25.Apr -29.Apr) range: 16.Apr-8.May	**20.5 days** (18-22) range:10-35
Quercus	**1892** (1858-2994) range:202-6045	**320** (310-521) range:14-1147	**12** (11-17) range:4-32	**26.Apr** (24. Apr -29.Apr) 15.Apr-10. May	**17.May** (14. May -20.May) range: 3.May-5.Jun	**18.5 days** (18-25) range:12-47
Poaceae	**3401** (3294-4154) range:2071-6348	**237** (235-320) range:116-586	**9** (8.6-11) range:6-18	**20.May** (17. May -21.May) range: 1.May-2.Jun	**19.Jul** (16.Jul -22.Jul) range: 2.Jul-9.Aug	**59.0 days** (58-65) range:47-81
Artemisia	**150** (133-206) range:20-471	**15** (14-24) range:5-66	**3** (2.5-3.5) range:1-7	**25.Jul** (22.Jul -26.Jul) range: 7.Jul-3.Aug	**22.Aug** (20. Aug -25.Aug) range: 14.Aug-8.Sep	**28.5 days** (28-34) range:19-55

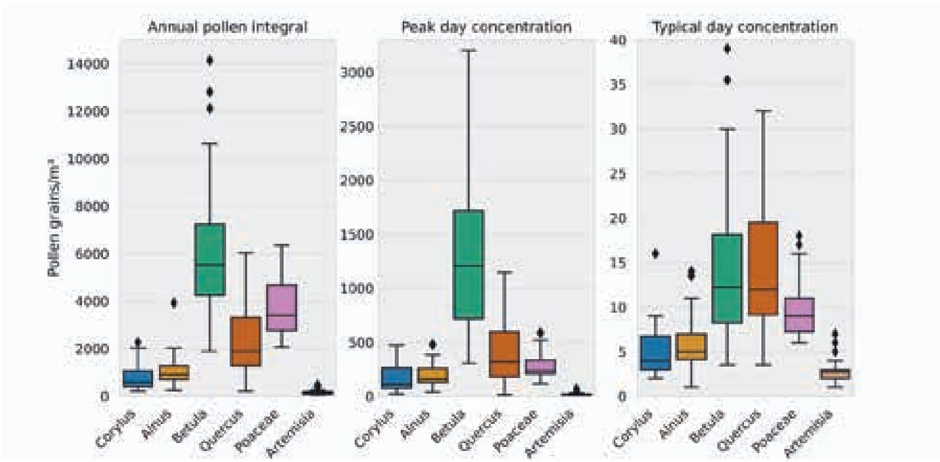
Season start, end and duration use the perc90 definition. For each characteristic, the median value of 30 seasons is given, followed by 95% confidence intervals of the median as determined by the bootstrapping method. The range indicates the minimum and the maximum value observed in the 30 seasons.

B. Pollen concentrations change in a highly dynamic manner

We analysed pollen emissions produced in an entire season and per day. Between 1991-2020, the median APIn for *Betula* was 5532 (95% CI: 5168-7262), but the total amount of pollen produced is highly variable between seasons, ranging from 1887 to 14134. Three *Betula* outlier seasons, defined as an APIn whose distance from the median APIn exceeds 1.5x the IQR, are indicated by diamonds in Fig 2. Also, the upper quartile of seasons (represented as the whisker at the top in the box-and-whisker plot) is wider than the lower quartile of seasons. This arrangement of outliers and quartiles implies that the distribution of *Betula* APIn is asymmetric and skewed towards very high values.

Characterisation of pollen concentrations

FIG 2.: *Quantification of pollen : for each of 30 seasons we determined the APIn (sum of daily concentrations over the entire season), peak day concentration (highest daily concentration in a season) and typical day concentration (median daily concentration in a season)*



To characterise daily observations within pollen seasons, we considered the peak day pollen concentration, as well as the concentration typical for an 'average' day within a season. For *Betula*, the peak day pollen concentration is on average 1209 pollen grains/m³ (median value of 30 seasons, 95% CI: 1105-1598). It has a wide range, from a minimum of 304 pollen grains/m³ in the season with the lowest peak day pollen concentration, to a maximum of 3200 pollen grains/m³ in the season with highest peak pollen concentration.

Thus a substantial portion of the birch APIn - over a fifth - may be produced in a single day, giving a measure of the most intense phase of the pollen season. To characterise pollen prevalence on a typical day of the season, which may be outside of the most intense period, we used the median pollen concentration, considering all of a season's days on which pollen was observed. For *Betula*, this value is on average 12 pollen grains/m³ (median of 30 seasons, 95% CI: 12-18), and ranges from 4 to 39 pollen grains/m³ in the 30 seasons analysed. Thus in the *Betula* season, pollen production can increase by two orders of magnitude from 12 pollen grains/m³ on a typical day to 1209 pollen grains/m³ on the peak day. Like the APIn, the distribution of the peak day pollen concentration and the typical day pollen concentration are both asymmetric and highly positively skewed in the 30 seasons.

The APIn of the three other trees we studied is lower than *Betula*, with median values of 568 pollen grains/m³ (95% CI: 618-1012) for *Corylus* ; 912 pollen grains/m³ (95% CI: 897-1378) for *Alnus* ; and 1892 pollen grains/m³ (95% CI: 1860-2998) for Quercus. Again, the range from the minimum to the maximum APIn is very wide between seasons, with a ~10-fold range from *Corylus* to a more than 30-fold range for Quercus.

The peak day pollen concentration of these trees is lower than that of *Betula* with median values of 112, 158, and 320 respectively for *Corylus*, *Alnus* and Quercus. The range of the peak day value is very wide, with the lowest and highest seasons varying from 14 to 1147 for Quercus.

The pollen concentration on a typical day is relatively close within tree species, with an average (median) of 4, 6, and 12 pollen grains per day respectively for *Corylus*, *Alnus* and Quercus. Again these values are highly variable from season to season, with, for example, Quercus ranging from a minimum typical day concentration of 4 to a maximum of 32 pollen grains per day within the set of 30 seasons.

The APIn for *Poaceae* is the second highest APIn after *Betula* with a median of 3401 pollen grains/m^3 (95% CI: 3294-4154). The *Poaceae* APIn is not as variable as the APIn of tree species, with a range of 2071 in the lowest season to a maximum APIn of 6348. The more extended *Poaceae* season is also less intense than *Betula*, with an average peak day of 237 pollen grains (95% CI: 235-320) but a typical day pollen concentration of 9 (95% CI: 9-11) .

With an average (median) APIn of 150 (95% CI: 133-206), *Artemisia* produces much smaller amounts of pollen than the other 5 species. The minimum to maximum APIn ranges from 20 to 471 pollen grains/m^3 in the 1991-2020 period studied. The average peak day pollen concentration of 15 pollen grains/m^3 (95% CI: 14-24) is substantially lower than in the other species. However, the pollen concentration on a typical day of the *Artemisia* season is more comparable to *Corylus* and *Alnus*, at 3 daily pollen grains/m^3 (95% CI: 2.5-3.5), with a minimum to maximum range of 1 to 7 daily pollen grains/m^3 in the 30 seasons.

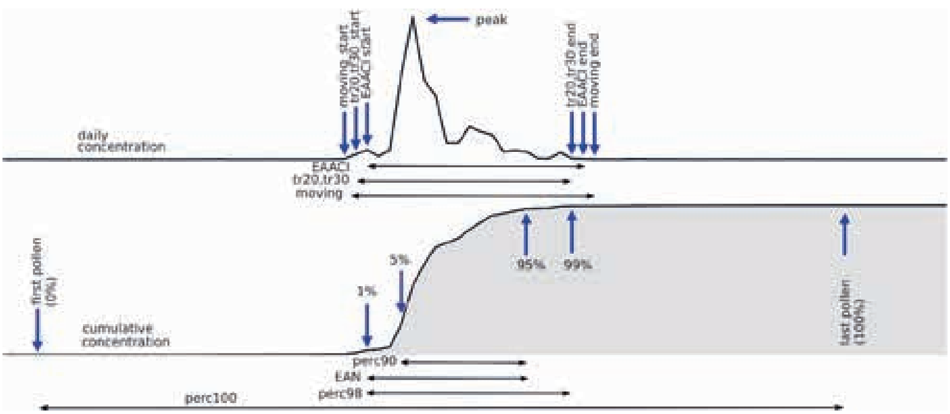
The ability of trees such as *Betula* or Quercus to increase their pollen production from a typical day value of 12 to 1200 (*Betula*) or 320 (*Quercus*) daily pollen grains/m^3 within just a few days is remarkable.

C. Season intensity and timing

The timing parameters of the pollen season include the days marking the start, end, and peak of the season, with the duration being the period from season start to season end. However there are multiple ways of defining the timing parameters and we have sought to compare the utility of these definitions. Threshold definitions of the pollen season consider the first and the last day on which the pollen concentration rises above a minimum concentration such as 20 (tr20, Fig 3.A) or 30 (tr30) pollen grains/m^3, or where the moving average of several days rises above a threshold (5,16,17). A drawback of these simple threshold definitions is that they do not account for the differences in APIn between taxa. For example, *Artemisia* pollen levels are too low to give meaningful results in Luxembourg with the tr20 and tr30 definitions (Fig 3). A more sophisticated definition set by the EAACI (15) considers both a daily threshold and a weekly threshold, at a level appropriate for the pollen type. A disadvantage of this definition is however that, while it exists for birch and grass pollen, it has not been specified for the other 4 plants in our study.

A second type of definition considers the period during which a given percentage of the APIn is observed, excluding the least intense days at the start and end of the season (7,14,16). For example, if 100% of the APIn is released between the first and last day on which pollen is observed (perc100, Fig 3.A), perc98 is the period between the days on which the 1. and 99. percentiles of that season's APIn are observed, excluding the days during which the first and the last percentile of the APIn are released. Similarly perc90 is the period between the days on which the 5. and 95. percentiles of the season's APIn are detected, excluding the least intense portions of the pollen season that account for the first 5% and the last 5% of the APIn. The EAN pollen database defines pollen seasons as the period between 1% and 95% of the API. An advantage of viewing the season as cumulative percentages of the APIn is that it automatically adjusts for differences in intensity between different pollen types, and between different years. A disadvantage is that it can only be determined retrospectively, once the season is over.

Comparison of perc100, perc98 and perc90 shows that excluding the first and last 1% or 5% of the APIn in the season definition reduces the season length considerably for all the taxa in our study. Similarly, the season-to-season variability of the season duration, measured as the IQR of the duration among the 30 seasons, implies that the perc90 definition produces more stable results in that the season timing varies by fewer days from season to season (Fig 3.B). The threshold definitions tr20 and tr30 give relatively compact seasons, comparable in length to the perc90 definition. However the season-to-season variability is higher than with perc90 for *Corylus* and *Alnus*, with an IQR of a magnitude similar to the median duration, though the variability is lower for *Betula*, *Quercus* and *Poaceae*. The variability of the tr20 and tr30 definitions is likely associated with how close the thresholds of 20 pollen grains/m³ and 30 pollen grains/m³ are to the 'typical day' pollen concentrations (Fig 2), which are around 5 pollen grains/m³ for *Corylus* and *Alnus*, but closer to 12 pollen grains/m³ for *Betula* and *Quercus*, and to 9 pollen grains/m³ for the long grass season. Importantly, the threshold definitions fail for *Artemisia* due to its low pollen concentrations, and, in the case of the EAACI definition, in addition also for the 3 trees other than birch.



In summary, each of the definitions for the season timing has its advantages and drawbacks. The first/last day of pollen is the most sensitive, while 5% percentile and threshold definitions are less variable. The EAACI definition is both robust to variability and clinically relevant, but is not defined for most of the pollen types in our study.

FIG 3*.A. Illustration of threshold and percentage definitions of pollen seasons, using a season of birch pollen concentrations (2015).*

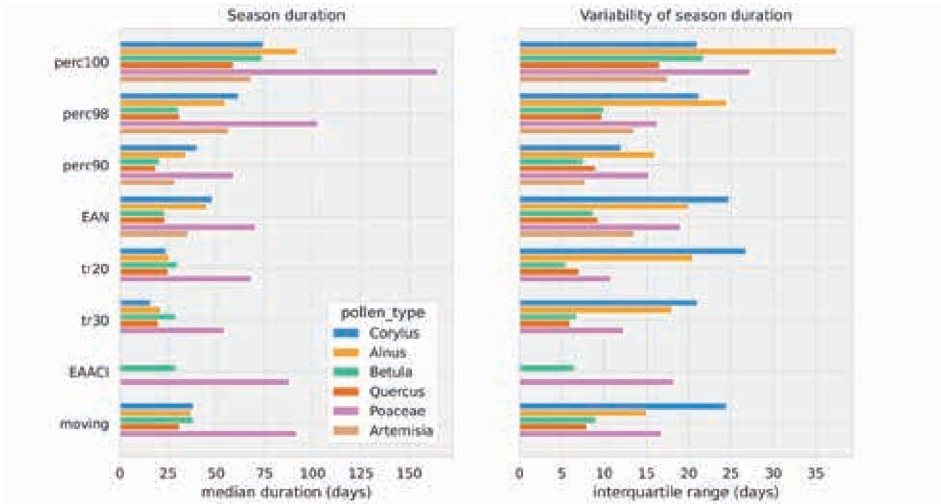


FIG 3.B. Comparison of season definitions. *Percentile and threshold season definitions were determined for each of the 6 allergenic taxa using the available seasons, and the median season duration is shown as a measure of magnitude, as well as the IQR as a measure of the variability between seasons.*

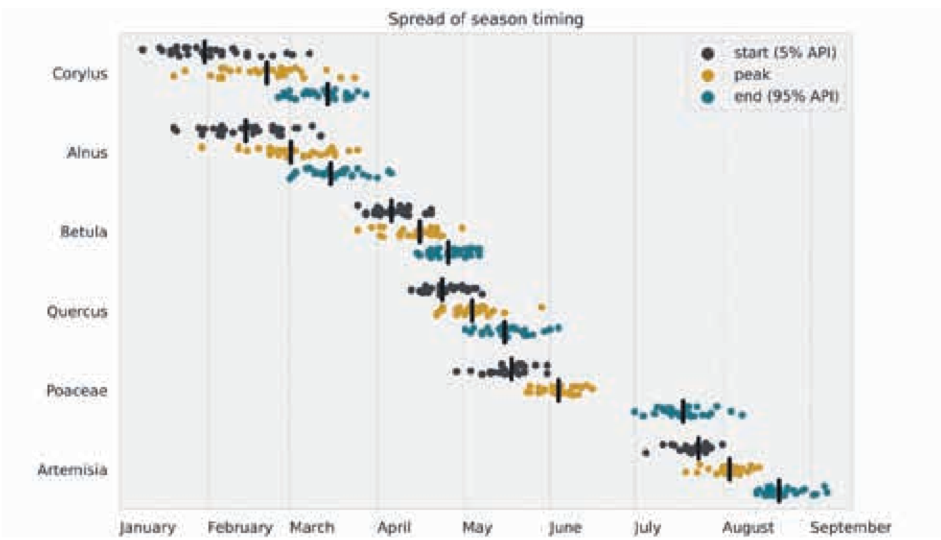


FIG 3.C.: Season timing of 6 allergenic taxa: *strip plot of season start and season end of 30 seasons according to the perc90 definition, as well as the peak day. The median of the 30 seasons is indicated.*

From 1991-2020, the *Corylus* season starts on average on 3. of February, (Table 1), and *Alnus* on 15. of February. The seasons of the other two tree species start in spring, typically on 8.April for *Betula*, and 26.April for *Quercus*. In comparison to the trees whose pollen season starts in winter, the *Betula* and *Quercus* seasons are both shorter. The *Poaceae* season typically starts around 20. of May, and lasts much longer than the tree seasons, with a median duration of 59 days. The *Artemisia* season starts typically on 25. of July, and lasts 28.5 days.

Comparing the spread of season timings (Fig 3.C), reveals that the most variable pollen season timing events occur earlier in the year. The *Corylus* and *Alnus* seasons may start in January, February or March, but almost always end in March, and the season start of the other 2 tree and 2 non-tree species is spread out over approximately 4 weeks between seasons. Also, the season duration varies 5-6 fold for the winter pollen seasons of *Corylus* and *Alnus*, but only 3-4 fold for the spring tree seasons *Betula* and *Quercus*, and 2-3 fold for the summer pollen seasons of *Poaceae* and *Artemisia*. This tendency is likely related to the lower temperatures when the catkins of *Corylus* and *Alnus* develop as well as the need to optimally time the pollen release with respect to the weather conditions which tend to be less favourable in winter.

D. Trend analysis over 30 years

Trend analysis reveals significant changes in pollen seasons over 3 decades

To gain insight into how pollen seasons have changed during the 1991-2020 period, we undertook trend analysis of the different aspects of the pollen seasons in this study. The main difficulty in establishing the existence of trends in pollen data is that the variability from one season to the next is generally large relative to the magnitude of the trend over 30 years. For this reason we used linear regression where possible as it has slightly higher statistical power ; and we used non-parametric methods (Mann-Kendall hypothesis test and Theil-Sen estimation) where the assumptions of linear regression such as constant variance, absence of serial correlation, and normality of residuals, were not met. In some cases, such as APIn, a linear trend fulfilling the assumptions of linear regression was revealed after logarithmic transformation. After fitting a trend line, the magnitude of the change was estimated as the difference between 1991 and 2020, expressed as the absolute change (e.g. the change in pollen grains/m^3), or as the relative change (e.g. the % increase of the 2020 value compared to 1991 fitted value). For measures that represent a day of the year, only the absolute change but not the percentage change is used.

D.1. Seasonal pollen concentrations

Trend analysis reveals increased tree APIn, but decreased herbaceous APIn from 1991 to 2020

Trend analysis of the APIn of *Corylus* pollen suggests that the hazel pollen produced in a season almost triples over the study period (FIG 4, Table S1). The trend line implies an expected APIn of 309 pollen grains/m³ for the year 1991 rises to 879 pollen grains/m³ in 2020, an increase of 184.5% (CI +51% to +448%) over three decades. This increase is statistically significant at a 5% significance level (p=1.7e-02, MK), though the large season-to-season variability leads to wide confidence intervals for the estimate. The peak pollen concentration and median pollen concentration also increase but their variability is too large to be statistically significant at a 5% level (Table S1). The *Alnus* APIn also increases between 1991 to 2020 from an expected value of 745 pollen grains/m³ to 1294 pollen grains/m³. However with a p-value of 8.8% (LR), this trend does not meet a 5% significance level.

The spring-flowering trees similarly display a trend toward higher APIn values: expected *Betula* APIn rise from 4161 pollen grains/m³ to 7549 pollen grains/m³ (p=4.6e-02, LR), and *Quercus* APIn from 1346 pollen grains/m³ to 2591 pollen grains/m³ (p=2.1e-01, LR) over the study period. Due to the large season-to-season variability, the confidence intervals for the trends are wide (see Table S1), and the trend from the *Quercus* data available is not statistically significant.

APIn trends 1991-2020.

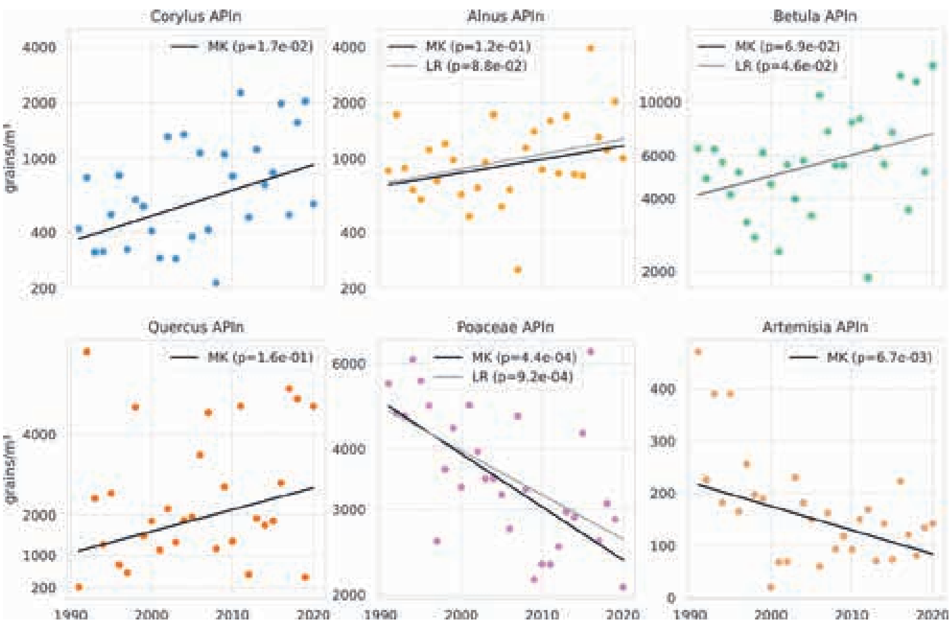


FIG 4: *Trends estimated for using linear regression (grey) where possible, and using Theil-Sen (black).*

Poaceae pollen shows a strong trend towards lower concentrations, with an APIn decreasing between 1991 and 2020 from 4795 to 2551 pollen grains/m³, the peak concentration decreasing 312 to 202 pollen grains/m³ (Table S1), and the typical day concentration from 12 pollen grains/m³ to 7 pollen grains/m³. Similarly, trend analysis implies that *Artemisia* pollen concentrations decrease between 1991 and 2020, with APIn value from 216 to 79 pollen grains/m^3, and peak concentration 27 to 8 pollen grains/m^3. The *Artemisia* APIn reduction is the stronger trend, with a reduction of 63.4% (CI: -118% to -22%, p=6.7e-03, MK), compared to the *Poaceae* APIn decrease of 46.8% (CI: -62% to -25%, p=9.2e-04, LR).

D.2 Season start, end, duration

Corylus *and* Alnus *season trends favour earlier season start*

The different measures of the start of the *Corylus* season show a trend towards earlier dates over the time frame of our study (Fig 5). For example, while the expected date of the first percentile of pollen (perc98 start) is the 2. of February in 1991, trend analysis implies that this is expected to occur 3 weeks earlier in 2020, on 12. of January (Table S1). Due to the large season-to-season variability, the confidence intervals of the trend estimates are wide, and not all p-values meet the 5% significance level. However, hazel pollination occurs 2-4 weeks earlier in 2020 compared to 1991, according to all the different definitions of the season start we used. Since each definition quantifies a distinct aspect of the start of the pollen season, the consistency of the trend makes it highly unlikely the observed earlier season start dates reflect random events, despite the wide confidence intervals.

In contrast to the trend towards an earlier start of the *Corylus* season, the corresponding season end is stable, with no statistically significant change in most definitions of the season end. According to the threshold definitions tr20 and tr30, the hazel pollination ends 7 respectively 11 days later, though this trend is not statistically significant (Table S1). As a result, the duration of the hazel season lengthens according to all definitions significant. For example, the period during which 98% of pollen is released (perc98) increases from 45 to 74 days (p=1.8e-02), while the period between the first and the last day on which hazel pollen concentrations surpass a threshold of 20 pollen grains/m^3 (tr20) increases from 14 to 43 days (p=9.9e-03).

Thus the trend is for the *Corylus* season to last much longer due to an earlier start, and since there is no decrease in the intensity of the season (peak pollen concentration and median pollen concentration tend to increase), the total pollen produced in each increases strongly, with a season APIn nearly tripling.

Like *Corylus*, the *Alnus* pollen season displays a trend to start at an earlier day of the year, while the end of the *Alnus* season is stable in most definitions (Fig 5). This results in a significantly longer duration of the season. For example, the expected perc98 start moves from 7. of February in 1991 to 17. of January in 2020 (p=4.7e-02) , increasing the perc98 duration from 44 to 68 days (p=4.3e-02) (Table S1). A difference between the two winter-flowering trees is, however, the trend of the peak concentration day: for *Corylus*, this tends to occur earlier, while it is delayed for *Alnus*.

Betula ***and*** **Quercus** ***seasons show a moderate trend to start earlier***

The *Betula* seasons tend to start slightly earlier according to different definitions (Fig 5). With a stronger trend towards earlier season end dates, the resulting season duration tends to be slightly shorter. However, the high variability between seasons makes these trends very uncertain. For example, according to the perc90

definition, the season starts 3.0 days earlier (9. of April to 6. of April , p=5.1e-01), ends 6.3 days earlier (30. of April to 24. of April , p=1.3e-01), resulting in a birch season expected to last 22 days in 1991 and 18 days in 2020 (p=4.2e-01). Similarly, according to the EAACI threshold definition, the birch season starts 3.7 days earlier, ends 6.1 days earlier, resulting in a season duration shortening from 30 days in 1991 to 28 days in 2020.

For the *Quercus* season, all measures of the season start and season end indicate a shift to occur several days earlier (Fig 5). Since this shift is very similar for start and end dates, the duration of the season is relatively unchanged. However, these trends are very uncertain since the statistical significance as expressed by p-values falls far short of the standard level of 5%. The strongest trend of the *Quercus* season timing regards the peak concentration, which is expected to occur on 12. of Mayin 1991, but on 27.April in 2020 (p=2.0e-03).

Trend analysis: absolute changes in season timing between 1991 and 2020

FIG 5. *The different definitions were used to determine season start, end, and duration. The absolute change (in number of days) between 1991 and 2020 is given as the difference between the two points in the trend line determined by linear regression if possible, otherwise Theil-Sen estimation, with the 95% confidence interval as indicated.*

Trends of Poaceae *and* Artemisia *season timing*

The *Poaceae* pollen season shows a trend to start earlier on all percentile definitions (Fig 5, Table S1). For example, the 5. percentile of pollen has a trend of occuring 10.2 days earlier (CI: -18 to -2 days, p=1.8e-02, LR), changing from 23. of May-to 13.May. There is no strong trend detectable for the end of the *Poaceae* pollen season when percentile definitions are used. However when threshold definitions are used, the season end does occur earlier. For example, the season according to the EAACI definition, is expected to end on 14. of August in 1991 but on to 26. of July in 2020, 18.5 days earlier (CI: -33 to -4, p=1.3e-02). This observation is linked to the general decrease in grass pollen concentrations, which means that the threshold is reached less often. For the same reason, while the period during which 98% of annual grass pollen are observed (perc98) increases from 94 to 111 days (+18.8 days, p=3.0e-02), the duration of the EAACI season is reduced from 97 to 82 days (-15.8 days, p=5.2e-02). The perc98 definition normalises for the decreasing APIn, while the lower intensity of grass pollen seasons means the threshold concentrations of the EAACI definition are exceeded on fewer days.

Unlike the other plants in our study, trends in the timing of the *Artemisia* pollen season are too weak to be statistically significant.

TABLE III. Summary of major trend changes over 30 years in pollen metrics

	Season start	End	Duration	Peak day	Peak concentration	APIn
Corylus	much earlier		much longer	earlier	higher	much higher
Alnus	much earlier	later	much longer	later		higher
Betula		earlier	shorter	earlier	higher	higher
Quercus	earlier	earlier		much earlier	higher	higher
Poaceae	earlier		longer/ shorter*	earlier	lower	lower
Artemisia				earlier	much lower	much lower

** longer according to the percentile definitions, shorter according to threshold (EAACI) definitions. For all details, see Table S1 (Annex)*

E. 30-Year trends of pollen seasons correspond to weather trends

A key determinant of pollen concentrations are the prevalent weather conditions. For example, the ambient temperature governs the rate at which tree flowers develop ; rainfall removes airborne particles, including atmospheric pollen (28,29). We therefore compared the pollen season trends we observed to weather trends over the same period. Although any correlation between the trends of weather and pollen seasons cannot by itself show any causal links, it nevertheless provides the context in which these trends can be interpreted.

First, we studied temperature and rainfall trends in the 1990-2020 period by aggregating data over each whole year. (We considered 1990 weather data as pollen seasons are influenced by the weather in the preceding year.) Trend analysis using a Mann-Kendall trend test suggests that the yearly mean temperature increased ($p=0.0065$) by 1.18°C between 1990 and 2020, from 8.66°C to 9.84°C (data not shown). A similar Mann-Kendall test of yearly rainfall found no trend ($p=0.63$) between 1990 and 2020, with a total yearly precipitation remaining around 880 l/m^2.

However, it is known that climate-change induced weather trends are not evenly spread throughout the year (30). Therefore we determined weather trends for each day of the year, calculating the absolute change between 1990-2020, considering the temperature over a 30-day window, and the precipitation in the previous 14 days. This analysis reveals that temperature increase is strongest in April (peak increase over 3°C), while the trend from late January to early March suggests little warming, and even some cooling (up to 1°C cooling). Interestingly, despite overall yearly precipitation showing no trend, February and August see increased precipitation (up to >12 l/m² increase in the preceding 14 days), while April and September display a trend towards decreased precipitation (reduction of >8 l/m²).

Change in temperature and precipitation, 1990-2020

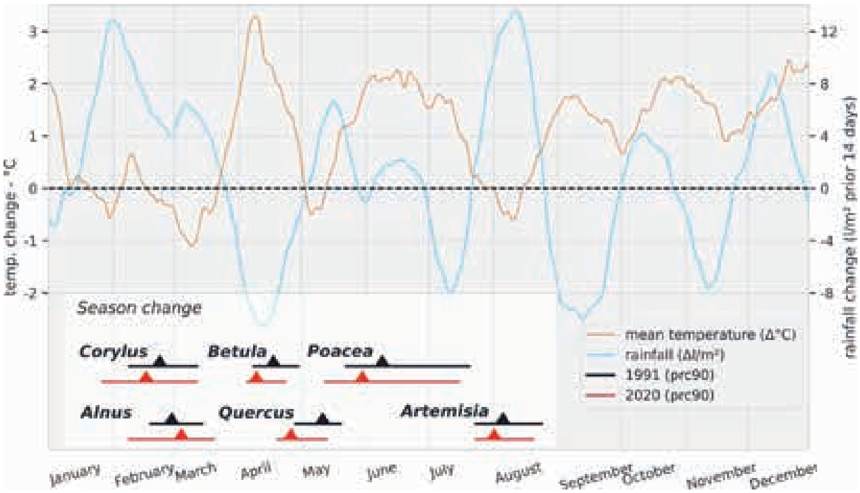


FIG 6. Trend analysis of temperature and precipitation: absolute changes 1990-2020. *The absolute change in mean temperature and precipitation was estimated for each day of the year. The timings of a typical season (peak day as a triangle, season start, end according to the perc90 definition) in 1991 (black) and 2020 (red) are also shown.*

The perc90 season duration and peak as expected for 1991 and 2020 according to the trends observed in the study period are shown in Fig 6, mapped on the temperature and precipitation trends. The main pollen season for *Corylus* and *Alnus* starts earlier. Hazel and alder catkins are known to develop in the weeks leading up to the start of the pollen season, i.e. particularly November and December. Thus the strong trend for earlier start dates in *Corylus* and *Alnus* pollen seasons is consistent with increased temperatures during the time in which the catkins develop (Fig 6). Conversely, the unchanged end dates of the *Corylus* season are associated with relatively unchanged temperatures during the hazel season ; while the delayed peak day and end of the *Alnus* season are consistent with a trend towards colder temperatures in the first half of March. The increased precipitation in mid-January to mid-March would be expected to have a negative impact on pollen concentrations, which is not observed.

The start of the *Betula* pollen season is largely determined by the temperature-dependent development of catkins, a process that initiates at the beginning of March. Similarly, higher temperatures accelerate the pollination process itself (28). Thus the trend towards a colder temperatures (up to -1°C) in the first half of March, followed by warmer temperatures in late March and particularly in April (up to >3°C) is consistent with observed changes in the *Betula* season: a weak trend towards an earlier season start in April, then a stronger trend towards earlier peak and season end. In addition, the reduction in precipitation in April is expected to promote higher pollen concentrations, consistent with the observation trend. In combination, these trends may contribute to a shorter, more intense *Betula* season. The changes in the *Quercus* season, which takes place 2-3 weeks later than the *Betula* season, are similarly consistent with the weather trends.

F. Impact on pollen allergic patients

Our trend analysis shows that the changes in pollen seasons are not uniform across taxa: for example, the *Poaceae* season has a lower APIn while lengthening its duration ; the *Betula* season has a higher APIn while shortening ; the *Corylus* season has higher APIn while increasing in duration (Table III). From the point of view of an allergy patient, the key aspect of a pollen season is the time period during which pollen concentrations are sufficiently high to cause allergic symptoms. To study how pollen season trends may affect allergic patients, we used plant-specific thresholds of concentrations likely to induce allergy symptoms. For each season the number of days with pollen concentrations below the allergy threshold (low pollen days), above the threshold (moderate pollen), and strong symptoms (high pollen) were determined (3,4). We also established the allergy period for each season, ie. the duration between the first and last day of pollen concentrations above the allergy symptom threshold, regardless of whether that period is interrupted by low/no pollen days, in line with the fact that once allergic symptoms are established, symptoms and mucosal inflammation take some time before resolution (39). Trend analysis was used to determine the absolute change (in days) from 1991 to 2020, as well as the relative change (in %), as shown in Table IV.

TABLE IV. *Changes in clinically relevant exposure, 1991-2020*

	low pollen days (sub-clinical levels)	**moderate pollen days (moderate symptoms)**	**high pollen days (strong symptoms)**	**allergy period**
Corylus	29 to 40 days +37% (-3% to +77%) p=7.2e-02 *	6 to 15 days +156% (+54% to +327%) p=7.6e-04 (LR, log) ***	2 to 5 days +194% (+0% to +484%) p=9.4e-02 (MK,TS) *	24 to 54 days +126% (+24% to +228%) p=1.7e-02 **
Alnus	28 to 40 days +41% (+0% to +83%) p=8.6e-02 (MK,TS) *	10 to 18 days +83% (+15% to +152%) p=1.9e-02 **	3 to 7 days +117% (-7% to +409%) p=7.2e-02 (LR, log) *	27 to 43 days +63% (-5% to +132%) p=6.7e-02 *
Betula	25 to 25 days +0% (-35% to +54%) p=9.9e-01 (LR, log)	15 to 9 days -39% (-78% to -0%) p=4.8e-02 **	14 to 18 days +23% (-8% to +55%) p=1.4e-01	37 to 31 days -14% (-44% to +15%) p=3.3e-01
Quercus	21 to 21 days +2% (-31% to +52%) p=9.1e-01 (LR, log)	11 to 14 days +24% (+0% to +67%) p=1.5e-01 (MK,TS)	9 to 12 days +44% (-35% to +123%) p=2.6e-01	24 to 34 days +40% (-5% to +84%) p=8.2e-02 *
Poaceae	43 to 59 days +37% (+10% to +63%) p=8.7e-03 ***	41 to 42 days +3% (-26% to +31%) p=8.4e-01	41 to 25 days -39% (-61% to -13%) p=4.5e-03 (MK,TS) ***	100 to 110 days +10% (-11% to +31%) p=3.4e-01
Artemisia	18 to 15 days -14% (-49% to +20%) p=4.1e-01	9 to 10 days +11% (-38% to +60%) p=6.5e-01	12 to 4 days -64% (-122% to -5%) p=3.4e-02 **	35 to 22 days -39% (-80% to +3%) p=6.7e-02 *

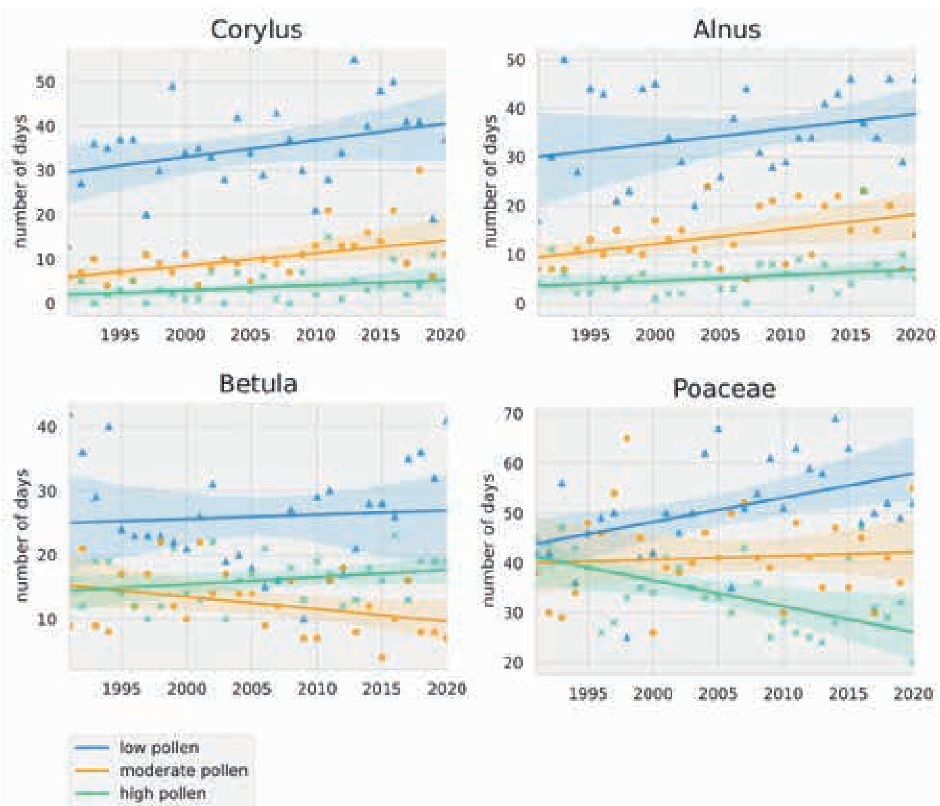
Absolute change (in days), and relative change (% change compared to 1991, including 95% CI), p-value of trend and trend analysis method used.

For *Corylus*, the trends for the number of low, moderate and high pollen days all increase between 1991 to 2020 (Fig 7, Table IV). The strongest trends are an increase in the number of moderate pollen days from 6 to 15 (p=7.6e-04), high pollen days from 2 to 5 (p=9.4e-02). The allergy period, which also includes intermittent days with low pollen, more than doubles in length, from 24 to 54 days (p=1.7e-02, Table IV). This is consistent with the observed trend of a lengthening of the hazel pollen season with a concomitant increase in APIn.

Trends of pollen concentrations according to clinical thresholds.

FIG 7. *Concentration on days in the low pollen category are unlikely to trigger symptoms in most patients, while days in the moderate and high pollen categories are sufficient to induce symptoms. The thresholds are taxon-specific, with moderate pollen thresholds 11-50/m³ for trees , 6-30/m³ for grass, 3-6/m³ for* Artemisia.

Consistent with the similar lengthening of the *Alnus* seasons, the clinically relevant trends for *Alnus* are very similar to those for *Corylus*, though the magnitude of the



changes is smaller, with moderate and high pollen days increasing from 10 to 18 days (p=1.9e-02) and 3 to 7 days (p=7.2e-02), respectively (Table IV).

For *Betula*, the number of moderate pollen days decreases (p=4.8e-02) by 39% from 15 to 9 days while the number of high pollen days increases (p=1.4e-01) 14 to 18 days and the number of low pollen days is stable at 25. This conversion of moderate pollen days into high pollen days (Fig 7) is consistent with the observation that a trend for the *Betula* season is becoming shorter while more pollen is produced overall. Interestingly, the overall effect of this intensification of the *Betula* pollen season is a 14% decrease in the period during which allergy sufferers are likely to experience symptoms, from 37 to 31 days. Thus the trend as experienced by *Betula* pollen allergy patients is likely to be relatively stable in comparison to the season-to-season variations, as expressed in the absence of a statistically significant trend in allergy period (p=3.3e-01).

For *Quercus*, the number of moderate and high pollen days both increase, and the allergy period lengthens by 40%, from 24 to 34 days (Table IV). However, the magnitude of these changes relative to the season-to-season variability results in lower statistical significance of these trends (p=8.2e-02 for the increase of the allergy period).

For *Poaceae*, the number of high-pollen days strongly decreases from 41 to 25 days (p=4.5e-03), the number of low-pollen days strongly increases from 43 to 59 days (p=8.7e-03), while moderate pollen days are stable (41, 42 days). This apparent conversion of high pollen days into moderate, and moderate into low pollen days (Fig 7) is consistent with our observation of a strong decrease in APIn with a simultaneous lengthening of the season. Perhaps surprisingly, the duration of the period in which either moderate or high pollen days are likely to cause allergic symptoms remains relatively stable, and shows an increase from 100 to 110 days that is statistically not significant (p=3.4e-01).

For *Artemisia*, the number of high pollen days is reduced by two-thirds, from 12 to 4 days (p=3.4e-02), and the allergy period shortens from 35 to 22 days (p=6.7e-02). This is consistent with the strong decreasing trend of *Artemisia* APIn while the duration of the season is relatively stable.

Discussion

Over the last 30 years the daily monitoring of atmospheric pollen concentrations in Luxembourg has been instrumental to inform allergic patients in a timely manner about the seasonal atmospheric “allergen load”. Indeed atmospheric pollen concentrations show great variability from day to day and also from year to year. Owing to the great amount of pollen data collected over this time period, we are now in a position to look for pollen trends and patterns over these years and put them in the context of weather parameters like temperature and rainfall.

When trying to gain an overview of the trends of allergenic pollen in Luxembourg, these are best understood in relation to the time of the year when pollination occurs, and whether the pollen is produced by trees or herbaceous plants. For the early flowering trees *Corylus* and *Alnus*, we detect a strong trend towards earlier and longer seasons. Pollination starts 3 weeks (perc98) earlier, and overall the pollen seasons lengthen by 29 and 24 days (perc98) respectively, over the 30 years analyzed (Table S1, Fig 5). At the same time, the *Corylus* APIn has nearly tripled (+184%) while the *Alnus* APIn has nearly doubled (+74%).

Similar increases in the APIn are seen with the spring flowering trees: we observe an 81% higher APIn for *Betula* and 93% higher for *Quercus* from 1991 to 2020. The season duration, which is shorter for the spring-flowering trees at 18, 20 days (perc90) compared to the winter-flowering trees with 34, 38 days, shows a slight shortening for *Betula* (4 days with perc90), while the duration is stable for *Quercus*. For the oak pollen season we find that the peak pollen day occurs at a significantly earlier time (17 days), and also has a tendency for an earlier season start (9 days, perc90). Thus all tree pollen concentrations show a strong increase and generally tend to occur earlier, while the winter- but not spring- pollen seasons lengthen.

In contrast to the deciduous tree, the pollen seasons of the two herbaceous plants in our study show a strong decrease in APIn, of 47% for *Poaceae* and 63% for *Artemisia* (Fig 4, Table S1). For the grass pollen season, we observed that it starts significantly earlier and the total duration of the season is longer, while there is little change in the timing of the *Artemisia* season.

Trend analysis of pollen season characteristics is complicated by the considerable variability between seasons. In order to detect a trend, analysis methods either require the magnitude of the trend to be large relative to the noise (ie. random season-to-season variability), or for pollen data to be available over a long time period. Since plants regulate pollination according to weather conditions, this variability largely reflects the variability of weather conditions, which particularly affects the timing of seasons. We used a variety of definitions to characterise the season timing. Since the different definitions capture different phenological aspects, the observation of consistent trends across the different definitions implies that these are not random events, but reflect genuine, underlying trends.

As changes in pollination are a response to changes in weather conditions, long-term pollen trends are thought to reflect long-term weather trends, i.e. climate change. For this reason we mapped the pollination trends we observed onto changes in temperature and precipitation (Fig 6). It is important to note that correlations between weather and pollen trends do not demonstrate a causal relationship as there may be other causes that could act indirectly or be wholly unrelated. In order to interpret pollen trends in the context of weather trends, it is essential to use botanical knowledge of pollination mechanics. As it is established that the rate of development of tree catkins is temperature-dependent, with higher ambient temperatures in the weeks preceding the pollen season bringing forward the start of pollination, it is likely that the trend towards higher temperatures in October, November and December contributes to the earlier start of *Corylus* and *Alnus* seasons. Similarly, the trend towards higher April temperatures is the likely reason for the *Quercus* season to occur earlier, while the trend for the first half of March to be colder likely prevents a corresponding earlier start of the *Betula* season. However, the relationship between weather conditions and pollination is complex, and in depth analysis will be needed for each individual species in relation to changes in temperature, rainfall, solar radiation to hopefully unravel the decisive forces at work. This task is beyond the scope of this paper.

These changes in pollination patterns have consequences for allergic patients. For those who are sensitized to *Corylus* and *Alnus*, the length of the allergy period increases, strongly in the case of *Corylus* and moderately for *Alnus*. For both taxa, the number of days with low, medium and high pollen concentrations show a significant increase or at least a tendency to increase. Concerning spring flowering trees, the number of days with medium *Betula* pollen concentrations shows a tendency to increase ; the oak pollen allergy period shows a tendency to lengthen (Table IV).

Importantly, all four trees in our study are members of the order of the Fagales (31) ; *Corylus*, *Alnus* and *Betula* belong to the family of the *Betula*ceae and *Quercus* to the family of Fagaceae. Members of this order share many allergens, the most important being the pathogenesis-related protein class 10 (PR-10), their major allergen, which includes a large group of cross reacting aeroallergens (32) and and common food allergens (33) responsible for the oral allergy syndrome (34).

For grass pollen, the large decrease in APIn and the lengthening of the season results in a decrease of the high pollen days which are converted into moderate pollen days. Perhaps surprisingly, the overall effect brings little relief for most grass allergy patients, since the period during which sufficient pollen is present to cause allergic symptoms is not reduced. By contrast, the *Artemisia* high pollen days decrease significantly and we observe a clear tendency for a shorter allergy period.

Earlier and longer pollen seasons together with higher APIn for early flowering pollen trees (*Corylus*, *Alnus*) , and to a lesser extend the springtime pollinating trees (*Betula*, *Quercus*), generally associated with decrease in APIn for herbaceous plants (*Poaceae*, *Artemisia*), sometimes associated with an earlier or longer pollen season have been reported by other authors in Belgium (6), the Netherlands (8), but also further off in Switzerland (5,7), Bavaria (35,36), Spain (37), and even for some tree pollen in North America (38).

These significant general observations in quite distant and very distant regions point to common phenomena at work and a link with climate change seems obvious. These trends in pollination have sometimes been linked to increases in temperature (5,6,8) or changes in rainfall. (37)

In Conclusion, the in depth analysis of 30 years of pollen recording in Luxembourg shows a significant trend for an earlier flowering of *Corylus* and *Alnus* combined with significant increase in total annual pollen concentrations. This lengthens the duration of time period of persons allergic to tree pollen, especially as there are important allergy cross-reaction between the pollen of *Corylus*, *Alnus*, *Betula* and *Quercus*. The grass pollen season starts earlier, the total annual concentration of grass pollen decreases significantly. For the moment no clear clinical improvement for the grass pollen allergic patient is to be seen, but this may change in the future if the *Poaceae* APIn falls further. In summary : over this 30 year period the situation for tree pollen allergic persons has worsened while there is a status quo but yet no clear cut improvement for hay fever patients to be observed even though grass pollen totals have a clear tendency to decrease.

Author contributions

data analysis was performed by PHe. PHe, FHa FHe drafted the first version of the manuscript. IP, MK, AH, MKB were responsible for the acquisition and maintenance of pollen data. All authors contributed to the revision and final approval of the manuscript.

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ANNEX

Table S1. *Trend analysis: absolute and relative change (1991-2020), p-value of trend*

	Corylus	*Alnus*	*Betula*	*Quercus*	*Gramineae*	*Artemisia*
APIn	309 to 879 +184.5% (CI: +51% to +448%) p=1.7e-02 (MK, TS) **	745 to 1294 +73.8% (CI: -8% to +230%) p=8.8e-02(LR, log) *	4161 to 7549 +81.4% (CI: +1% to +225%) p=4.6e-02(LR, log) **	1346 to 2591 +92.5% (CI: -32% to +445%) p=2.1e-01(LR, log)	4795 to 2551 -46.8% (CI: -62% to -25%) p=9.2e-04(LR, log) ***	216 to 79 -63.4% (CI: -118% to -22%) p=6.7e-03 (MK, TS) ***
typical day conc.	4 to 6 +58.1% (CI: -13% to +187%) p=1.3e-01(LR, log)	5 to 7 +22.0% (CI: -50% to +94%) p=5.3e-01	13 to 11 -13.5% (CI: -94% to +65%) p=6.5e-01 (MK, TS)	12 to 16 +26.7% (CI: -52% to +105%) p=4.9e-01	12 to 7 -43.5% (CI: -61% to -19%) p=3.0e-03(LR, log) ***	3 to 3 +0.0% (CI: -55% to +0%) p=4.3e-01 (MK, TS)
peak conc.	62 to 167 +170.0% (CI: -36% to +510%) p=8.7e-02 (MK, TS) *	145 to 171 +18.0% (CI: -47% to +161%) p=6.7e-01(LR, log)	733 to 1718 +134.4% (CI: -25% to +235%) p=8.7e-02 (MK, TS) *	214 to 385 +79.7% (CI: -53% to +586%) p=3.8e-01(LR, log)	312 to 202 -35.3% (CI: -62% to +9%) p=1.0e-01(LR, log)	27 to 8 -69.9% (CI: -85% to -39%) p=1.6e-03(LR, log) ***
peak day	23.Feb to 16.Feb -6.5 days (CI: -27 to +14) p=5.1e-01	28.Feb to 5.Mar +4.7 days (CI: -12 to +21) p=5.7e-01	18.Apr to 10.Apr -8.1 days (CI: -19 to +4) p=1.7e-01(LR, log)	12.May to 27.Apr -15.2 days (CI: -24 to -6) p=2.0e-03 ***	9.Jun to 31.May -9.5 days (CI: -16 to -3) p=9.0e-03 ***	6.Aug to 2.Aug -4.3 days (CI: -10 to +1) p=1.4e-01 (MK, TS)

perc100 start	8.Feb to 3.Jan -36.5 days (CI: -38 to -31) p=2.6e-05(LR, log) ***	24.Jan to 30.Dec -26.3 days (CI: -42 to -11) p=1.8e-03 ***	28.Mar to 17.Mar -10.4 days (CI: -23 to +2) p=9.7e-02 *	10.Apr to 8.Apr -1.6 days (CI: -12 to +9) p=7.7e-01	22.Apr to 29.Mar -24.1 days (CI: -45 to -3) p=2.6e-02 **	9.Jul to 15.Jul +6.0 days (CI: -7 to +19) p=3.2e-01 (MK, TS)
perc98 start	2.Feb to 12.Jan -20.5 days (CI: -28 to -4) p=2.5e-02(LR, log) **	7.Feb to 17.Jan -21.3 days (CI: -42 to -0) p=4.7e-02 **	4.Apr to 4.Apr +0.2 days (CI: -9 to +9) p=9.6e-01	23.Apr to 17.Apr -6.3 days (CI: -17 to +4) p=2.3e-01	14.May to 29.Apr -14.4 days (CI: -23 to -6) p=1.6e-03 ***	16.Jul to 20.Jul +4.1 days (CI: -13 to +12) p=5.7e-01 (MK, TS)
perc90 start	8.Feb to 26.Jan -12.9 days (CI: -32 to +6) p=1.8e-01	18.Feb to 8.Feb -10.1 days (CI: -29 to +8) p=2.7e-01	9.Apr to 6.Apr -3.0 days (CI: -12 to +6) p=5.1e-01	29.Apr to 20.Apr -8.6 days (CI: -17 to +0) p=7.3e-02 (MK, TS) *	23.May to 13.May -10.2 days (CI: -18 to -2) p=1.8e-02 **	24.Jul to 24.Jul +0.0 days (CI: -9 to +4) p=7.1e-01 (MK, TS)
EAACI start	-	-	6.Apr to 2.Apr -3.7 days (CI: -13 to +6) p=4.2e-01	-	8.May to 5.May -2.9 days (CI: -13 to +7) p=5.5e-01	-
tr20 start	16.Feb to 26.Jan -21.3 days (CI: -41 to -1) p=3.8e-02 **	21.Feb to 11.Feb -9.4 days (CI: -29 to +10) p=3.3e-01	3.Apr to 1.Apr -1.6 days (CI: -11 to +7) p=7.2e-01	27.Apr to 21.Apr -6.0 days (CI: -14 to +6) p=1.5e-01 (MK, TS)	16.May to 8.May -8.6 days (CI: -17 to +0) p=5.4e-02 *	-
tr30 start	22.Feb to 31.Jan -21.4 days (CI: -44 to +1) p=5.8e-02 *	23.Feb to 17.Feb -6.3 days (CI: -24 to +12) p=4.8e-01	3.Apr to 2.Apr -1.7 days (CI: -10 to +7) p=7.0e-01	28.Apr to 21.Apr -7.9 days (CI: -16 to +5) p=1.0e-01 (MK, TS)	18.May to 14.May -3.2 days (CI: -13 to +6) p=4.9e-01	-
moving start	16.Feb to 19.Jan -28.8 days (CI: -53 to -5) p=2.0e-02 **	17.Feb to 5.Feb -11.5 days (CI: -30 to +7) p=2.2e-01	2.Apr to 31.Mar -2.7 days (CI: -12 to +7) p=5.7e-01	25.Apr to 18.Apr -7.7 days (CI: -15 to +6) p=8.7e-02 (MK, TS) *	9.May to 1.May -8.6 days (CI: -18 to +0) p=5.9e-02 *	-
perc100 end	31.Mar to 29.Mar -1.7 days (CI: -14 to +11) p=7.8e-01	5.Apr to 2.May +26.7 days (CI: +3 to +50) p=2.7e-02 **	7.Jun to 2.Jun -4.5 days (CI: -23 to +14) p=6.2e-01	11.Jun to 5.Jun -6.0 days (CI: -15 to +3) p=1.8e-01	25.Sep to 23.Sep -2.2 days (CI: -7 to +3) p=3.7e-01	22.Sep to 22.Sep +0.0 days (CI: -10 to +7) p=7.3e-01 (MK, TS)
perc98 end	20.Mar to 22.Mar +2.4 days (CI: -10 to +15) p=6.9e-01	26.Mar to 25.Mar -1.3 days (CI: -14 to +11) p=8.4e-01	11.May to 29.Apr -11.5 days (CI: -20 to -3) p=1.4e-02 (MK, TS) **	27.May to 21.May -5.6 days (CI: -15 to +4) p=2.5e-01	16.Aug to 19.Aug +3.2 days (CI: -10 to +16) p=6.2e-01	11.Sep to 17.Sep +5.3 days (CI: -10 to +18) p=3.8e-01 (MK, TS)
perc90 end	13.Mar to 13.Mar -0.1 days (CI: -12 to +11) p=9.8e-01	15.Mar to 20.Mar +5.4 days (CI: -7 to +17) p=3.6e-01	30.Apr to 24.Apr -6.3 days (CI: -14 to +2) p=1.3e-01(LR, log)	20.May to 14.May -6.7 days (CI: -17 to +4) p=2.0e-01	21.Jul to 16.Jul -5.0 days (CI: -16 to +6) p=3.6e-01	25.Aug to 21.Aug -3.9 days (CI: -12 to +5) p=3.7e-01(LR, log)

EAACI end	-	-	6.May to 30.Apr -6.1 days (CI: -15 to +2) p=1.6e-01	-	14.Aug to 26.Jul -18.5 days (CI: -33 to -4) p=1.3e-02 **	-
tr20 end	5.Mar to 13.Mar +7.5 days (CI: -11 to +27) p=3.2e-01 (MK, TS)	11.Mar to 20.Mar +9.0 days (CI: -5 to +23) p=2.0e-01	4.May to 30.Apr -4.0 days (CI: -12 to +4) p=3.2e-01	19.May to 16.May -3.4 days (CI: -10 to +11) p=5.3e-01 (MK, TS)	25.Jul to 10.Jul -14.3 days (CI: -24 to -5) p=5.5e-03 ***	-
tr30 end	26.Feb to 9.Mar +11.1 days (CI: -8 to +30) p=2.4e-01	10.Mar to 19.Mar +8.8 days (CI: -5 to +23) p=2.1e-01	2.May to 29.Apr -2.6 days (CI: -12 to +6) p=5.6e-01	17.May to 10.May -6.5 days (CI: -16 to +3) p=1.9e-01	15.Jul to 6.Jul -9.2 days (CI: -18 to -1) p=3.3e-02 **	-
moving end	14.Mar to 16.Mar +1.6 days (CI: -15 to +18) p=8.5e-01	17.Mar to 25.Mar +7.7 days (CI: -4 to +20) p=2.1e-01	13.May to 3.May -9.3 days (CI: -17 to -1) p=2.5e-02 **	24.May to 22.May -1.4 days (CI: -11 to +8) p=7.5e-01	13.Aug to 26.Jul -18.2 days (CI: -32 to -4) p=1.2e-02 **	-
perc98	45 to 74 +66.2% (CI: +12% to +120%) p=1.8e-02 **	44 to 68 +52.0% (CI: +2% to +102%) p=4.3e-02 **	37 to 26 -31.5% (CI: -59% to -4%) p=2.8e-02 **	32 to 33 +3.9% (CI: -29% to +52%) p=8.4e-01(LR, log)	94 to 111 +18.8% (CI: +2% to +36%) p=3.0e-02 **	58 to 60 +4.0% (CI: -31% to +38%) p=8.2e-01
perc90	33 to 45 +39.2% (CI: -14% to +92%) p=1.4e-01	25 to 40 +62.2% (CI: +2% to +122%) p=4.3e-02 **	22 to 18 -15.4% (CI: -54% to +23%) p=4.2e-01	18 to 19 +8.9% (CI: -32% to +68%) p=6.8e-01 (MK, TS)	58 to 64 +9.4% (CI: -10% to +33%) p=3.6e-01(LR, log)	31 to 29 -5.2% (CI: -33% to +34%) p=7.5e-01(LR, log)
perc100	60 to 93 +54.1% (CI: +25% to +83%) p=7.9e-04 ***	62 to 136 +118.1% (CI: +76% to +161%) p=5.9e-06 ***	71 to 77 +8.4% (CI: -21% to +38%) p=5.7e-01	59 to 59 +0.0% (CI: -25% to +28%) p=9.9e-01 (MK, TS)	157 to 178 +14.0% (CI: -1% to +29%) p=6.2e-02 *	74 to 66 -11.1% (CI: -35% to +12%) p=3.4e-01
EAACI	-	-	30 to 28 -8.0% (CI: -30% to +14%) p=4.6e-01	-	97 to 82 -16.2% (CI: -30% to +0%) p=5.2e-02(LR, log)	-
tr20	14 to 43 +213.5% (CI: +55% to +372%) p=9.9e-03 ***	16 to 36 +122.4% (CI: +0% to +234%) p=5.8e-02 (MK, TS) *	32 to 29 -7.7% (CI: -33% to +18%) p=5.4e-01	22 to 28 +27.6% (CI: -15% to +70%) p=1.9e-01	69 to 63 -8.3% (CI: -26% to +9%) p=3.4e-01	-
tr30	4 to 38 +747.1% (CI: +169% to +2567%) p=7.1e-04(LR, log) ***	15 to 30 +100.8% (CI: -2% to +203%) p=5.3e-02 *	29 to 28 -3.1% (CI: -32% to +26%) p=8.3e-01	18 to 22 +18.2% (CI: +0% to +78%) p=3.0e-01 (MK, TS)	59 to 53 -10.3% (CI: -31% to +11%) p=3.2e-01	-
EAN	36 to 62 +74.5% (CI: +15% to +134%) p=1.6e-02 **	35 to 60 +74.1% (CI: +17% to +131%) p=1.3e-02 **	26 to 20 -24.9% (CI: -58% to +8%) p=1.4e-01	25 to 25 +0.7% (CI: -39% to +66%) p=9.8e-01(LR, log)	68 to 77 +13.8% (CI: -6% to +33%) p=1.6e-01	41 to 34 -17.8% (CI: -43% to +19%) p=2.8e-01(LR, log)

moving	26 to 56 +118.2% (CI: +12% to +224%) p=3.0e-02 **	27 to 48 +75.7% (CI: +22% to +165%) p=4.9e-02 (MK, TS) **	40 to 34 -16.3% (CI: -39% to +6%) p=1.5e-01	29 to 36 +24.1% (CI: -8% to +56%) p=1.4e-01	96 to 86 -10.4% (CI: -33% to +9%) p=3.2e-01 (MK, TS)	-
low pollen days	29 to 40 +36.9% (CI: -3% to +77%) p=7.2e-02 *	28 to 40 +41.1% (CI: +0% to +83%) p=8.6e-02 (MK, TS) *	25 to 25 +0.3% (CI: -35% to +54%) p=9.9e-01(LR, log)	21 to 21 +2.3% (CI: -31% to +52%) p=9.1e-01(LR, log)	43 to 59 +36.6% (CI: +10% to +63%) p=8.7e-03 ***	18 to 15 -14.1% (CI: -49% to +20%) p=4.1e-01
moderate pollen days	6 to 15 +156.2% (CI: +54% to +327%) p=7.6e-04(LR, log) ***	10 to 18 +83.5% (CI: +15% to +152%) p=1.9e-02 **	15 to 9 -38.9% (CI: -78% to -0%) p=4.8e-02 **	11 to 14 +24.4% (CI: +0% to +67%) p=1.5e-01 (MK, TS)	41 to 42 +2.8% (CI: -26% to +31%) p=8.4e-01	9 to 10 +10.8% (CI: -38% to +60%) p=6.5e-01
high pollen days	2 to 5 +193.5% (CI: +0% to +484%) p=9.4e-02 (MK, TS) *	3 to 7 +117.3% (CI: -7% to +409%) p=7.2e-02(LR, log) *	14 to 18 +23.3% (CI: -8% to +55%) p=1.4e-01	9 to 12 +44.4% (CI: -35% to +123%) p=2.6e-01	41 to 25 -39.3% (CI: -61% to -13%) p=4.5e-03 (MK, TS) ***	12 to 4 -63.8% (CI: -122% to -5%) p=3.4e-02 **
allergy period	24 to 54 +126.3% (CI: +24% to +228%) p=1.7e-02 **	27 to 43 +63.5% (CI: -5% to +132%) p=6.7e-02 *	37 to 31 -14.5% (CI: -44% to +15%) p=3.3e-01	24 to 34 +39.5% (CI: -5% to +84%) p=8.2e-02 *	100 to 110 +9.8% (CI: -11% to +31%) p=3.4e-01	35 to 22 -38.6% (CI: -80% to +3%) p=6.7e-02 *

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Vaccine effectiveness in preventing SARS-CoV-2 infections and COVID-19 hospitalisations and deaths in elderly adults aged 70 years and older, Luxembourg, June 2021

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Rapid communication

ABSTRACT

We evaluated vaccine effectiveness (VE) against three outcomes among elderly ≥ 70 years, using data from the COVID-19 surveillance system in Luxembourg. The deployed vaccines provide good protection, particularly among fully vaccinated individuals against SARS-CoV-2 infections (84.6% [95%CI: 79.1-88.6]), hospitalizations (98.9% [95%CI: 91.9-99.9]), and deaths (91.9% [95%CI: 76.9-97.2]). Comirnaty® is the strongest contributor to these promising outcomes. Post-first dose Vaxzevria® prevented 69.1% [95%CI: 52.8-79.8] of infections. A similar substantial VE was also observed in residents of long-term care facilities (LTCFs).

Keywords: COVID-19; SARS-CoV-2; Luxembourg, elderly people; Long-term care facilities; vaccine-effectiveness

Introduction

The greatest burden of SARS-CoV-2 has been observed among the elderly people, resulting in excessive COVID-19-related deaths in many countries, including Luxembourg(1).

When the vaccination programme initiated on 28th December 2020, the Luxembourgish government prioritised elderly people (64 567 individuals), including those living in LTCFs, to reduce the risk of COVID-19-related morbidity and mortality in this (vulnerable) population, accounting for almost 10% of the population in Luxembourg (634 163 residents, STATEC, 1st January 2021).

In early 2021, Luxembourg experienced an increase in the incidence of COVID-19 per 100,000 habitants, particularly among residents of LTCFs, compared to non-institutionalised subjects of the same age group, despite the implementation of stringent non-pharmaceutical interventions (NPIs). This observation was concomitant with the occurrence of SARS-CoV-2 outbreaks in some nursing homes in January and March 2021. This resurgence of new cases was associated with two peaks in COVID-19-associated hospitalisations and deaths, among the institutionalised subjects.

The real-world effectiveness of SARS-CoV-2 vaccines in older adults is uncertain(2). More data are needed, particularly in LTCFs residents, against severe forms of the disease, leading to hospitalization and death(3).

Our aim was to investigate the protective effect of first and second dose of overall and product-specific COVID-19 vaccines, in elderly aged ≥ 70 years, with a focus on LTCFs residents. We estimated the effectiveness of the deployed vaccines, namely: Comirnaty® (BNT162b2, BioNTech-Pfizer, Mainz, Germany/New York, United States); Vaxzevria® (ChAdOx1-S, AstraZeneca, Cambridge, United Kingdom); COVID-19 Vaccine Janssen® (Ad26.COV2-S; Janssen-Cilag International NV, Beerse, Belgium) and Spikevax® (CX-024414, Moderna Cambridge, United States), in preventing SARS-CoV-2 infection, hospitalisation and death over time since vaccination.

Information sources, study design and setting

The Ministry of Health ensures the surveillance of COVID-19 pandemic, via a national monitoring system collecting epidemiological and vaccination data. A daily reporting of PCR test results by all diagnostic laboratories and of COVID-19-associated admissions and deaths from hospitals and LTCFs is mandatory. This vaccine effectiveness analysis relies on a daily longitudinal linkage of the micro-data from epidemiological surveillance platform, national vaccination and mortality registries, using pseudonymised patients' identification numbers. The data analysed in the present paper was delivered by the IGSS in a secured virtual environment whilst being compliant with the GDPR regulation.

In this observational study, we analysed nationwide surveillance data, from the start of the vaccination programme on 28th December 2020 until 15th June 2021, to assess the effectiveness of the deployed vaccines against various SARS-CoV-2 outcomes. The study population consisted of individuals aged ≥70 years, including LTCFs residents.

Statistical analysis

We compared the incidence of SARS-CoV-2 in vaccinated subjects at different intervals after vaccination. As immunity develops during the first 14 days after vaccination, the incidence of disease during these few days approximates that of a non-vaccinated group and is therefore, considered as a reference(4). VE is calculated by comparing the incidence during this "0-13 days" interval with the incidence during different non-overlapping intervals post vaccination, namely 14-20; 21-27 and ≥ 28 days following vaccination with the first and second dose.

Person-days are calculated as the sum of time each person included in the study is at risk. The numerator refers to the first manifestation of the disease (first positive PCR test), while the denominator is equal to the sum of all disease-free times during the period chosen for the incidence calculation. The attack rate is defined as the number of cases during the at-risk period divided by the person-days. Cases of hospitalization and death are linked to the date of the corresponding PCR test.

VE was computed as a percentage: (1 - (attack rate in vaccinated/attack rate in “reference group”)) x 100.

95% confidence intervals were calculated to determine precision. All statistical analyses were performed with RStudio® (version 4.0).

Ethical statement

The planning, conduct and reporting of the study was in line with the Declaration of Helsinki. Official ethical approval and patients consents were not required, as data collection is part of the national pandemic surveillance system set-up under the authority of the Ministry of Health.

Vaccine uptake

Our analysis included 51 844 individuals ≥70 years and 4 734 LTCFs residents; showing a vaccination uptake of 80% among ≥70 year olds and 84% among LTCFs residents by June 15th 2021. Among both groups, 97% of individuals vaccinated had received their second dose at the time of analysis.

LTCFs residents were older (median age 87 years [IQR 82; 91] vs. 77 years [73; 83]), and included more women (76% vs. 56%). Among subjects ≥70 years, 31 703 (61%) received Comirnaty®, 18 379 (35%) Vaxzevria®, 1 480 (3%) Spikevax® a and 282 (1%) Janssen® vaccine. Almost the entire LTCFs population was vaccinated with Comirnaty® (Table 1).

Table 1 *Characteristics of the vaccinated individuals aged 70 years and older and residents in LTCFs (institutionalised), included in the analysis according to their vaccination status, Luxembourg, 28 December 2020 to 15 June 2021*

	Vaccinated ≥ 70 years subjects (N = 51 844)	Vaccinated institutionalised subjects (N = 4 734)
Age, years		
Median [IQR]	77 [73;83]	87 [82;91]

Sex		
Male	22 556 (43.51%)	1 122 (23.70%)
Female	29 288 (56.49%)	3 612 (76.30%)
Vaccine uptake*	51 844 / 64 567 (80%)	4 734 / 5 627 (84%)
Vaccination status		
First dose only	1 677 (3.23%)	143 (3.03%)
Second dose	50 167 (96.77%)	4 591 (96.97%)
Administered vaccine		
Comirnaty	31 703 (61.15%)	4 716 (99.7%)
Vaxzevria	18 379 (35.45%)	2 (0.04%)
Spikevax	1 480 (2.85%)	7 (0.15%)
Janssen	282 (0.54%)	9 (0.19%)
Outcomes**		
PCR test positive	587 (1.13%)	251(5.30%)
Hospitalisation	103 (0.19%)	29 (0.61%)
Death	62 (0.12%)	41 (0.87%)
*Defined as the proportion of individuals vaccinated with at least one dose until June 15th 2021 among the eligible population (residents 70 years and older). ** Number of cases documented from 28th December 2020 to 15th June 2021.		

According to the national vaccination programme, the institutionalised people were vaccinated steadily earlier than the other elderly aged ≥70 years (Figure 1).

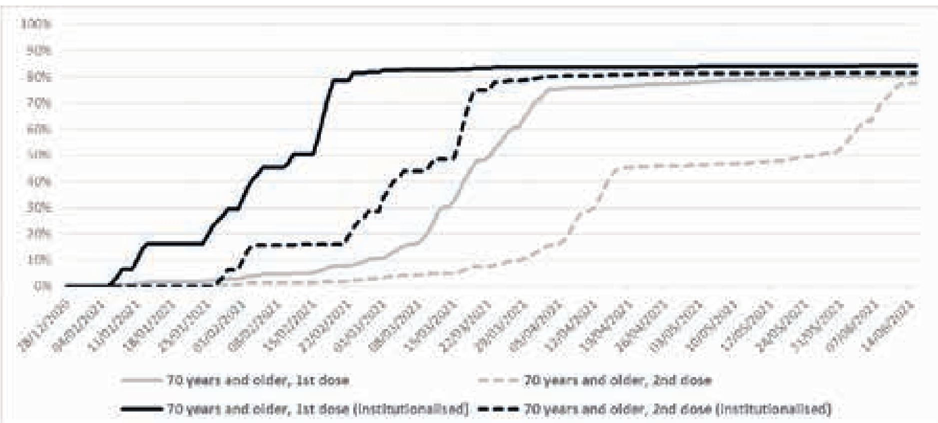


Figure 1 *Vaccination uptake over time of the first and second doses in people aged 70 years and older, and in LTCFs residents, 28 December 2020 to 15 June 2021*

Vaccine effectiveness

The proportion of PCR positive cases was higher (5.3%) among LTCFs residents, compared to all-elderly people ≥70 years (1.1%) (Table 1).

The incidence of infections per 10,000 person-days decreased gradually over the fixed intervals in subjects ≥70 years, offering moderate protection against infection (68% [95%CI: 58.5-76.1]), hospitalization (78% [95%CI: 58.9-88.6]) and death (80.6% [95%CI: 52.5-92.1]) at least 28 days following first dose vaccination (Table 2A).

After full vaccination, VE increased to 84.6% [95%CI: 79.2-88.6] against infection, 98.9% [95%CI: 91.9-99.9] against hospitalization, and 91.9% [95%CI: 76.9-97.2] against death (Table 2A). A single-dose Janssen® vaccine, with only one positive PCR test case, registered 28 days post vaccination, was excluded from the analysis.

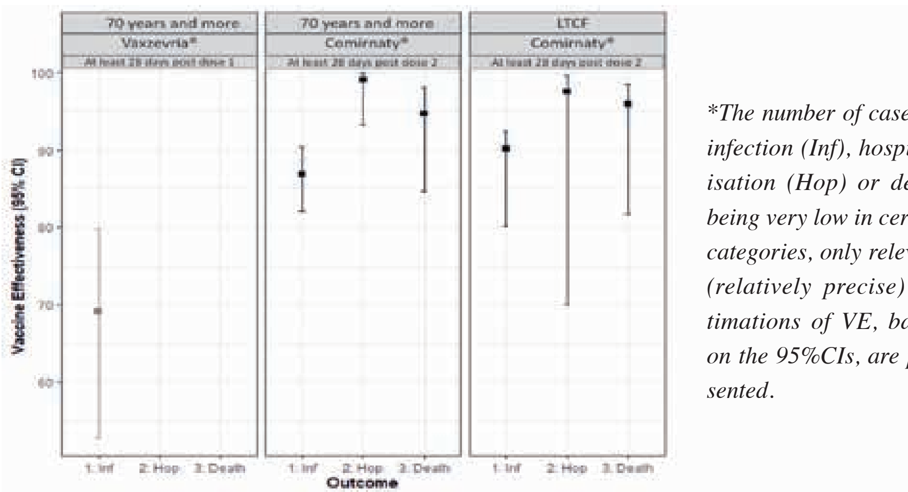
Among the institutionalized persons, a higher VE, starting from 28 days post second dose is observed against SARS-CoV-2 infections (90.1% [95%CI: 85.3- 93.4]), hospitalisation (97.8% [95% CI: 82.6- 99.7]) and death (95.9% [95%CI: 87.8-98.6]) (Table 2B).

		After first dose				After second dose			
		Interval since vaccination				Interval since vaccination			
		0-13 days	**14-20 days**	**21-27 days**	**≥28 days**	**0-13 days**	**14-20 days**	**21-27 days**	**≥28 days**
Table 2A Among all subjects 70 years and older									
	Persons-days (p-d)	668 373	358 672	351 290	860 313	577 106	232 579	220 776	1 386 560
Infection	Cases*	175	85	78	71	75	26	20	56
Infection	Incidence per 10,000 p-d	2.62	2.37	2.22	0.83	1.30	1.12	0.91	0.40
Infection	VE	reference	9.49 (-17.28; 30.15)	15.20 (-10.74; 35.06)	68.48 (58.47; 76.08)	50.37 (34.95; 62.13)	57.30 (35.54; 71.72)	65.40 (45.05; 78.22)	84.57 (79.16; 88.58)
Hospitalisation	Cases*	43	19	17	12	7	3	1	1
Hospitalisation	Incidence per 10,000 p-d	0.64	0.53	0.48	0.14	0.12	0.13	0.05	0.01
Hospitalisation	VE	reference	17.66 (-41.29; 52.01)	24.78 (-31.89; 57.10)	78.32 (58.89; 88.57)	81.15 (58.09; 91.52)	79.95 (35.37; 93.78)	92.96 (48.87; 99.03)	98.88 (91.86; 99.85)
Death	Cases*	24	11	12	6	4	1	0	4
Death	Incidence per 10,000 p-d	0.36	0.31	0.34	0.07	0.07	0.04	0.00	0.03
Death	VE	reference	14.59 (-74.36; 58.16)	4.87 (-90.23; 52.43)	80.58 (52.49; 92.06)	80.70 (44.37; 93.30)	88.03 (11.49; 98.38)	100.00 (-,-)	91.97 (76.85; 97.21)
Table 2B Among institutionalised subjects									
	Persons-days (p-d)	**61 407**	33 040	27 714	18 495	59 646	32 078	32 039	352 382
Infection	Cases*	67	30	30	16	41	11	18	38
Infection	Incidence per 10,000 p-d	10.89	9.07	10.82	8.64	6.87	3.43	5.62	1.08
Infection	VE	reference	16.78 (-28.00; 45.90)	0.79 (-52.60; 35.50)	20.71 (-36.79; 54.04)	37.00 (7.08; 57.29)	68.57 (40.54; 83.39)	48.51 (13.36; 69.40)	90.12 (85.28; 93.36)
Hospitalisation	Cases*	8	4	4	3	7	1	1	1
Hospitalisation	Incidence per 10,000 p-d	1.30	1.21	1.44	1.62	1.17	0.31	0.31	0.03
Hospitalisation	VE	reference	7.02 (-208.61; 72.02)	-10.79 (-267.92; 66.64)	-24.51 (-369.32; 66.97)	9.92 (-148.42; 67.33)	76.07 (-91.33; 97.01)	76.04 (-91.56; 97.00)	97.82 (82.58; 99.73)

Death	Cases*	17	7	6	4	3	0	0	4
	Incidence per 10,000 p-d	2.76	2.12	2.16	2.16	0.50	0.00	0.00	0.11
	VE	reference	23.47 (-84.54; 68.26)	21.80 (-98.35; 69.17)	21.88 (-132.17; 73.71)	81.83 (38.00; 94.68)	100.00 (-;-)	100.00 (-;-)	95.90 (87.81; 98.62)
* Cases refer to the number of people who tested positive, were hospitalised, and died of SARS-CoV-2, during a given time period (28 December 2021 to 15 June 2021).									

***Table 2 A&B** Incidence of COVID-19 in vaccinated persons aged 70 years and older by interval since vaccination and estimated vaccine effectiveness (95% CI), by comparing incidence during the 0-13 days interval with incidences during other intervals (Section A: among all subjects 70 years and older; Section B: among institutionalised subjects)*

Comirnaty® vaccine effectiveness increased progressively to 86.9% [95%CI: 82.0-90.4], 99.1% [95%CI: 93.3-99.9], and 94.7% [95%CI: 84.7-98.2] against infection, hospitalization and death, respectively, ≥28 days after the second dose. Vaxzevria® VE against infections was 69.1% [95%CI: 52.8-79.8] after a single dose, whereas no hospitalisations nor deaths occurred after two doses (Figure 2).



**The number of cases of infection (Inf), hospitalisation (Hop) or death being very low in certain categories, only relevant (relatively precise) estimations of VE, based on the 95%CIs, are presented.*

***Figure 2** Vaccine effectiveness [95% CI]* among all elderly ≥70 years including institutionalised subjects by type of vaccine, Luxembourg, 28 December 2020 to 15 June 2021*

Discussion

Our results suggest a promising effectiveness of most used vaccines in Luxembourg (Comirnaty® and Vaxzevria®) in preventing SARS-CoV-2 infections, reducing hospitalisations and deaths, among elderly adults, including LTCFs residents – a more vulnerable population, and a primary target for the national vaccination programme.

Consistent with other studies(3), (5), (6), our findings confirmed an encouraging VE after full vaccination, suggesting that a second dose provides valuable additional protection. An immunosenescence phenomenon could explain the longer time required to observe vaccine protection among this population(7).

Our study has several strengths. It suggests a different design based on calculating the "person-time incidence rate" across different intervals since vaccination, which allows attenuating exposure bias (4). It provides nationwide estimates of product-specific effectiveness after partial and complete vaccination, against a range of outcomes.

Major limitations include the observational nature, and lack of information on the symptomatic/asymptomatic status of PCR tested patients, and their comorbidities and the fact that exposure could have been different between reference and comparator groups.

Although it is complex to distinguish the impact of the vaccination program from that of existing NPIs, during the observation period, it is noteworthy that the decrease in SARS-CoV-2 incident cases was concomitant with the increase in vaccination coverage, suggesting that the main factor in this reduction might be related to the effectiveness of the vaccines used.

These initial results are of immediate relevance to our national authorities and for formulation or adjustments of current vaccination strategies, in different countries using these vaccines.

Authors' contribution

AA: Conceived, designed the analyses and drafted the manuscript. SS: Analysed the data and revised the manuscript. SL, MD, GW, JM, FB: contributed to the critical and intellectual revision of the manuscript.

FB: involved in the coordination of data collection; TD: contributed to the acquisition of data and preparation of the secured platform for the analyses. All authors approved the final version.

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Hémophilie A : Evolution thérapeutique

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Introduction

L'hémophilie A est une maladie congénitale due à une mutation du gène q28 du facteur VIII (FVIII) qui se trouve sur le chromosome X. Cette perte d'activité de ce facteur de la coagulation se solde par un taux de FVIII faible dans le sang et une insuffisance de coagulation avec risques de saignements. Le taux normal est de 50-150%. L'hémophilie A est classée en trois groupes suivant le taux de FVIII, à savoir : FVIII sanguin <1%, hémophilie sévère, 1-5% hémophilie modérée, 5-40% hémophilie mineure. Ces taux reflètent aussi la gravité de la maladie et l'indication thérapeutique correspondante.

Dans l'hémophilie sévère les patients ont des problèmes majeurs de saignement en général mais surtout au niveau des articulations et des muscles, souvent après des traumatismes mineurs. Les patients qui par le passé, lors du diagnostic ne pouvaient bénéficier des traitements de remplacement de FVIII ont encore aujourd'hui des séquelles articulaires ayant parfois nécessité des interventions orthopédiques de correction. Dans l'hémophilie modérée il peut y avoir des problèmes hémorragiques après traumatismes plus important, lors d'extractions dentaires et en chirurgie en général.

Traitement

Le traitement de l'hémophilie A a été longtemps peu efficace et limité à des attelles, à l'immobilisation et à des sacs de glace avec une espérance de vie de moins de 20 ans…

Les premiers traitements étaient par transfusion de sang, peu efficaces, dépendant d'hospitalisations et de l'infrastructure sanitaire correspondant à l'époque. Suite au fractionnement du sang, le sang complet a été remplacé par du plasma. Ces traitements étaient destinés à traiter des saignements et il n'y avait pas d'utilisation de prévention thérapeutique. Dans les années 1950 la technique du cryoprécipité du plasma a permis d'avoir un extrait plus riche en FVIII et un traitement plus spécifique est devenu possible.

Traitement par FVIII.

Par après l'extraction du FVIII du plasma a nettement améliorée le traitement. Ces produits sont rapidement devenus disponibles. Toutefois des problèmes de transmission d'agents infectieux, hépatite C et HIV, ont fait la une des journaux dans les années 1980 : « le scandale du sang contaminé ». Dans beaucoup de pays les FVIII administrés étaient souvent contaminés avant l'arrivée des tests du SIDA en 1985. Très rapidement, vers 1983 aux Etats-Unis, un producteur majeur de FVIII, Travenol, a soupçonné qu'il pouvait y a voir un problème de contamination, a retiré tous ses produits du marché et distribué un produit chauffé, heat treated (HT FVIII) ayant donc éliminé le virus HIV et également celui de l'hépatite C. Sur-ce le CDC (Center of Disease Control) américain a recommandé de limiter les FVIII aux produits chauffés. Ces produits sont devenus un standard thérapeutique avec une nette amélioration de la situation des patients. Suivant le producteur d'autres techniques de purification tout aussi efficaces sont utilisées (S/D solvant detergent…) Avec l'étape suivante, à savoir le génie génétique, un facteur VIII recombinant (rFVIII), produit stérile, est arrivé sur le marché avec une excellente tolérance et sécurité. Les traitements au moindre signe de possible saignement sont devenus standard et également suivant les patients une attitude de prévention est devenue possible avec une nette diminution des problèmes articulaires. L'auto-administration intraveineuse, par le patient ou un parent à domicile ou en milieu sanitaire a constitué une nette amélioration de la qualité de vie des patients.

La demi-vie des FVIII standard est de 10-14h. Mais au cours de la dernière décennie la demi-vie des rFVIII a pu être prolongée à environ 48h et plus grâce à diverses techniques telles que la pégylation, la fusion de rFVIII avec un domaine de monomère d'IgG1 (rFVIII-Fc) et autres. De ce fait ces produits ont une demi-vie prolongée (extended half-life EHL) permettant de réduire la fréquence des administrations de 2 à 3 fois par semaine dans le cadre de la prévention et tout aussi efficaces en traitement de saignements. Ce type de traitement est devenu le standard dans la plupart des pays où la situation économique le permet

Il faut noter que 20 à 30% des patients souffrant d'hémophilie A auront un problème d'anticorps anti FVIII. Le traitement de ces patients est difficile. Il faut saturer l'anticorps par l'administration massive de FVIII avec de bons résultats mais à des coûts prohibitifs. Ce traitement présente les mêmes résultats avec les différents FVIII disponibles. Une autre façon de traiter ces situations est l'administration de produits induisant la coagulation en court-circuitant le FVIII tel le complexe activé de prothrombine ou le Facteur VII recombinant activé (rFVIIa).

En ce moment la situation thérapeutique est satisfaisante avec des produits EHL permettant un traitement en situation stable de 2-3 administrations intraveineuses par semaine le but étant d'avoir un taux de >1% de FVIII sanguin au nadir, c'est-à-dire juste avant l'administration suivante. Ainsi les patients ont un taux de saignements annuel bas avec 2/3 des patients sans épisode de saignement au cours d'une année et une espérance de vie proche de celle de la population masculine normale.

Les inconvénients restent l'administration intraveineuse dépendant de la qualité de l'accessibilité veineuse surtout chez les enfants et nécessitant souvent la mise en place d'un accès veineux central.

L'administration sous-cutanée de FVIII a été essayé dans une étude préliminaire avec des résultats intéressants (1). L'avantage est évidemment la qualité de vie du patient, sans injections intraveineuses, sans accès veineux central, une plus grande autonomie du patient et probablement une meilleure adhérence. L'inconvénient peut être le volume nécessaire à administrer surtout chez les enfants. Pour ce type d'administration il n'y a actuellement pas encore de données suffisantes pour sa généralisation.

Traitements de non remplacement de FVIII.

La situation a changé pour un certain nombre de patients en 2017 avec la mise sur le marché d'un produit non basé sur le remplacement du FVIII.

- **Emicizumab :** commercialisé par Roche sous la dénomination Hemlibra. Il s'agit d'un anticorps monoclonal bi-spécifique qui imite la fonction du FVIII en faisant une liaison entre le FIX activé et le FX ainsi court-circuitant le FVIII déficitaire. Il faut signaler que ce produit a une demi-vie longue et les études cliniques ont montré une protection contre saignement correcte suite à une administration dans des protocoles divers allant de 1x par semaine à 1x par mois et ceci en sous-cutané auprès de patients avec ou sans anticorps anti-FVIII, déjà prétraités ou non par FVIII (2,3). L'emcizumab a toutefois divers inconvénients. L'effet n'est pas mesurable par un test de laboratoire ce qui est un inconvénient dans l'établissement du dosage d'administration. En cas de saignement ou de chirurgie, il faudra recourir à une administration de FVIII standard. Il n'est pas clair non plus pour le moment s'il faut adapter le dosage d'emcizumab par rapport au degré d'activité du patient.

Une intéressante évaluation de l'utilisation de Hemlibra en Europe a montré dans les centres évalués une utilisation large du produit avec un traitement jusqu'à 100% des patients avec anticorps inhibiteur du FVIII et jusqu'à 88% des patients sans inhibiteur. Un taux de saignements annuels de zéro a été atteint auprès de 73% des patients. Dans l'ensemble après trois années de mise sur le marché en Union Européenne, le produit est largement disponible avec une excellente efficacité et une sécurité satisfaisante (4).

D'autres produits de non remplacement de FVIII sont à l'étude :

- **Le Fitusiran.** Ce produit diminue la production de l'antithrombine et a donc un effet procoagulant. Les études préliminaires ont montré un excellent résultat avec une administration jusqu'à toutes les quatre semaines auprès de patients sans et avec anticorps anti FVIII. En cas de saignements les traitements avec un FVIII, FVIIactivé et APCC ont été efficace. Des études phase III sont actuellement en cours (5)

- **Le Concizumab.** est un anticorps anti TFPI, procoagulant. Une étude phase III est en cours (6)

Thérapie génique : en hémophilie A le gène codant pour le FVIII est défectueux et le but thérapeutique est de remplacer par un vecteur adénoviral non pathogène avec un gène fonctionnel inséré capable de produire de manière continue du FVIII fonctionnel à un taux modéré et suffisant pour éviter un supplément par du FVIII exogène. Ces séquences d'ADN transportées par des vecteurs recombinant adénovirus peuvent avoir une expression prolongée dans divers tissus dont notamment le foie d'où les protéines FVIII produites peuvent facilement pénétrer la circulation. Des études sont en cours dont quelques unes confirment un bénéfice au long cours avec des résultats très intéressants avec un minimum, voire même zéro saignement par an (7). Il faudra attendre des compléments de résultats, notamment de durée et l'avenir nous dire quand et quelle en sera l'applicabilité de routine.

- **Genome editing :** des techniques de « genome editing » sont à l'étude et vont peut-être voir le jour à l'avenir. Actuellement une étude phase I est en cours dans l'hémophilie B (8). Pour le moment il n'y a que des données à court terme. Il faudra attendre la suite d'études en cours, la stabilité du modèle et les résultats à long terme.

Conclusion :

Au cours des dernières décennies, la mise à disposition de FVIII recombinant a d'abord rendu ces produits plus sûr du point de vue infectieux que les produits antérieurs. L'arrivée de FVIII à demi-vie prolongée (EHL) a amélioré la situation du traitement préventif par des injections de 2-3 fois par semaine. Plus récemment les produits non de remplacement du facteur VIII ont complètement changé la situation surtout pour les patients présentant des anticorps et difficiles à traiter. Actuellement un seul produit, le Hemlibra est commercialisé en Union Européenne, mais d'autres produits de non remplacement sont à l'étude. Le traitement par génie génétique est très prometteur et sera probablement disponible encore dans la décennie actuelle.

Remarque : En dehors du problème d'hémostase, il est possible que le FVIII ait d'autres fonctions au long cours. Il est intéressant de noter que 20 à 30% des patients hémophiles souffrent d'ostéoporose et une hypothèse de cause à effet a été soulevée par l'intermédiaire du RANK ligand (9)

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