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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Impact of time-varying confounders on the association between early-life allergy sensitization and the risk of current asthma: A post hoc analysis of a birth cohort

To the Editor,

Existing literature on the relationship between early-life (first year of life) allergy sensitization and risk of childhood asthma is mixed.[1,2] This is in part due to the use of statistical analytic methods that ignore changes in both allergy sensitization status and asthma-related treatment exposure that may influence future asthma risk as a child grows older. Our recent disease transition models show that both childhood allergy sensitization and current asthma states (and plausibly related treatment) are time-varying, and the likelihood of a feedback loop cannot be ruled out[3]; this underscores the analytic challenges associated with evaluating to what extent early-life allergy sensitization may be causally related to childhood asthma development. Moreover, it is unclear if early-life allergen avoidance prevents or merely delays the onset of current asthma into adolescence or adulthood.[4]

In contrast to traditional discrete-time and longitudinal models (e.g., Generalized Estimating Equations—GEE) used in previous research,[1,2] Marginal Structural Models (MSM) can be used to adjust for time-varying confounding to produce consistent average causal effect estimands.[5] Previous simulation studies have demonstrated the superiority of the MSM over traditional longitudinal statistical methodology.[5] In this study, we hypothesize that among infants genetically predisposed to asthma, early-life allergy sensitization is associated with an increased risk of current asthma but this risk can be attenuated by allergen avoidance. We carried out a post hoc analysis using the MSM approach to estimate the average causal effect of early-life allergy sensitization and allergen avoidance on the risk

of current asthma under the context of dynamic (changing) allergy sensitization-current asthma states in the Canadian Asthma Primary Prevention Study (CAPPS).[6] CAPPS was a multifaceted intervention designed to decrease exposure in the first year of infancy to indoor aeroallergens such as house dust mites and pets and to encourage prolonged breastfeeding and delayed introduction of milk and solid foods.[6] Current asthma and allergy sensitization were based on a pediatric allergist's clinical decision and skin prick test results, respectively, at age 1-, 2-, 7-, and 15-years. Briefly, MSMs are estimated using an inverse-probability-of-treatment (or exposure) weighted approach[5] to remove the effects of time-varying confounders (i.e., post-baseline allergy sensitization and asthma-related treatment states) in the pathway between early-life sensitization and subsequent risk of current asthma (see Appendix S1 for details).

The prevalence of current asthma and allergy sensitization varied during follow-up with and without CAPPS exposure (Figures 1, S1 and S2). The prevalence of current asthma did not differ between children sensitized to aeroallergens vs. food allergens in the first 2 years; however, during the 7th and 15th years, the odds of current asthma were six and four-fold higher among children sensitized (vs. un-sensitized) to food and aeroallergens, respectively (Figure S2). These results suggest different profiles of allergy sensitization (define by type and age) may differentially influence or modify the propensity of school-age asthma development. Our MSM model results (Table 1) showed that the odds of current asthma were higher among children with (vs. without) an early-life allergy sensitization (adjusted odds ratio [aOR]: 3.02; 95% CI: 1.51, 6.01) at age 7-years;

Abbreviations: aOR, adjusted Odds Ratio; CAPPS, Canadian Asthma Primary Prevention Study; CI, confidence intervals; GEE, Generalized Estimating Equations; IPTW, inverse-probability-of-treatment (or exposure) weighted approach; MSM, Marginal Structural Models; OR, Odds Ratio.
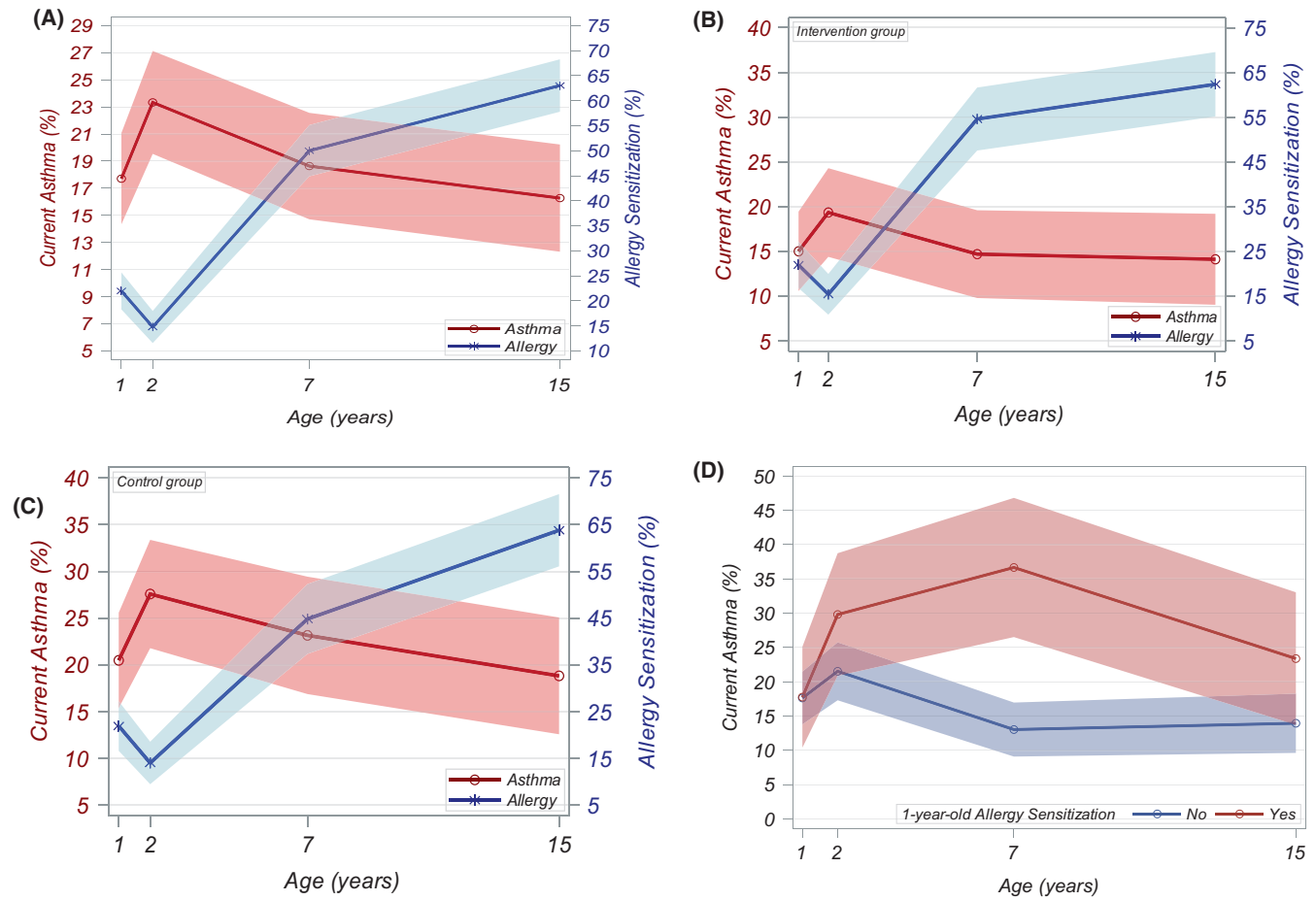
FIGURE 1 Prevalence of current asthma and allergy sensitization during a 15-year follow-up among children in the CAPPS cohort with 95% Confidence Interval Bands (N = 525). (A) Overall Study Sample; (B) Intervention Group; (C) Control Group; (D) Early-life (1st year) allergy sensitization and current asthma

however, no differences were observed at 15-years (aOR: 2.32; 95% CI: 0.78, 6.85). In contrast, the GEE model results overestimated the strength of this association by 49% at 7-years (aOR: 4.50; 95% CI: 2.53, 8.00) and incorrectly estimated its precision at 15-years (aOR: 2.03; 95% CI: 1.02, 4.04). Overall, the odds of current asthma were lower among children randomized to the CAPPS intervention (aOR: 0.69; 95% CI: 0.51, 0.93). Female children had 28% lower odds of current asthma than male children (aOR: 0.72; 95% CI: 0.53, 0.98).

Our results suggest that early life is an etiologically critical window during which allergy sensitization may induce pathogenesis towards school-age asthma onset irrespective of whether the pattern of sensitization is transient or persistent. Conversely, allergen avoidance during this period may reduce the risk of current asthma. Discrepancies between our GEE and MSM results for these competing risk and protective effects on current asthma suggest that confounding due to time-varying allergy sensitization states and asthma-related treatment exposure may explain some of the null associations reported in previous research.[1,2] This underscores the need to adjust for time-varying confounders when investigating the association between current asthma and suspected risk and protective factors under dynamic risk contexts.

Our findings (i.e., allergy sensitization—current asthma induction period and early-life as a critical etiologic window with intervention potential) may be generalizable to both developed and less developed countries; however, interpretation of similar analyses in these heterogenous populations should not preclude an examination of potential effect moderation/modification by environmental factors. In the absence of randomized baseline exposures (e.g., allergy sensitization), MSMs hold promise for the elucidation of childhood asthma causal mechanisms needed to inform the timing and strategies for preventive intervention.

TABLE 1 Generalized Estimating Equation (GEE) and Marginal Structural Model (MSM) results of the association between early-life allergy sensitization, CAPPS intervention, and current asthma

	GEE 1[a]		MSM-1[b]		MSM-2[c]	
	OR (95% CI)	p value[e,f]	OR (95% CI)	p value[e,f]	OR (95% CI)	p value[e,f]
Early-life allergy sensitization (ref = No)						
Year 1	1.29 (0.65, 2.55)	.469	1.01 (0.59, 1.75)	.967	1.01 (0.59, 1.75)	.966
Year 2	1.32 (0.73, 2.40)	.363	1.58 (0.90, 2.79)	.111	1.54 (0.87, 2.72)	.134
Year 7	**4.50 (2.53, 8.00)**	**<.0001**	**2.87 (1.46, 5.64)**	**.002**	**3.02 (1.51, 6.01)**	**.002**
Year 15	**2.03 (1.02, 4.04)**	**.004**	1.91 (0.71, 5.19)	.201	2.32 (0.78, 6.85)	.127
CAPPS intervention (ref = Control)[d]	**0.63 (0.44, 0.88)[g]**	**.008**	**0.70 (0.53, 0.93)[g]**	**.016**	**0.69 (0.51. 0.93)[g]**	**.014**
Sex (ref = Male)	0.73 (0.52, 1.04)	.077	**0.72 (0.53, 0.97)**	**.035**	**0.72 (0.53, 0.98)**	**.040**
Birth mode (ref = Cesarean section)	0.71 (0.48, 1.03)	.091	0.80 (0.55, 1.16)	.236	0.82 (0.54, 1.25)	.358
Gestational age	0.88 (0.76, 1.01)	.086	0.89 (0.79, 1.01)	.072	0.89 (0.77, 1.02)	.092
Low birth weight (ref = No)	1.37 (0.78, 2.41)	.435	1.28 (0.13, 13.05)	.834	1.27 (0.12, 12.98)	.842
Asthma treatment (ref = No)	**1.91 (1.39, 2.63)**	**<.001**				

[a]GEE model 1 with an independent working correlation structure. The association between early-life allergy sensitization and current asthma at each follow-up year is adjusted for CAPPS intervention assignment, all time-invariant factors (sex, birth mode, gestational age, and low birth weight), and time-varying asthma treatment.

[b]MSM-1 results are adjusted for all time-invariant factors (sex, birth mode, gestational age, and low birth weight), time-varying allergy sensitization, and missing data (# multiple imputations = 20).

[c]MSM-2 results are adjusted for all time-invariant factors (sex, birth mode, gestational age, and low birth weight), time-varying allergy sensitization, time-varying asthma-related treatment exposure, and missing data (# multiple imputations = 20).

[d]CAPPS intervention effect did not differ across time (CAPPS x Time: p value =.686 in the GEE-1).

[e]p values were captured from Type 3 analysis.

[f]p values comparing early allergy sensitization status and CAPPS intervention at each follow-up on current asthma risk were obtained from pairwise comparison.

[g]Average CAPPS intervention effect across years on current asthma.

Bold values are statistically significant at $p < 0.05$.

KEYWORDS

allergy sensitization, childhood asthma, marginal structural models

CONFLICT OF INTEREST

All authors have no financial conflict of interest and nothing to disclose.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Immune-inflammatory proteome of elite ice hockey players before and after SARS-CoV-2 infection

To the Editor,

Coronavirus disease 19 (COVID-19) is an infectious disease transmitted mainly through aerosol spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and in most cases leads mild to moderate respiratory illness, which usually resolves within 5–7 days.[1] Regular moderate-to-vigorous exercise has been associated with a strong and timely immune response against infections, thus reducing susceptibility to acute respiratory illness, and also protecting from severe COVID-19 outcomes.[2,3] Frequent high-intensity training has also been proposed to enhance vaccine-induced cellular and humoral immunity.[2] However, long-term high-intensity physical activity and stressors associated with elite sports might cause hyperinflammation in some individuals and increase the risk of respiratory illness, and ice hockey players are among those winter sport athletes, who have the highest incidence in that context.[3,4] Proteomic profiling of COVID-19 patients has proven valuable in the discovery of novel biomarkers associated with disease susceptibility, course, complications, and severity,[5] but so far there are no reports of COVID-19 proteomic studies in athletes. Herein, we examined the immune-inflammatory proteome of elite ice hockey players before and after a team-wide COVID-19 outbreak with the omicron BA.1 variant in December 2021.

Serum blood samples and questionnaire data were obtained from 24 players of a Swiss National League ice hockey team 3 months prior to COVID-19 and from the same players within 1–2 weeks after nasal swab PCR-confirmed SARS-CoV-2 infection, and of 20 controls, that are non-ice hockey players after recent recovery from COVID-19. Written informed consent was obtained from all study participants,

and the protocol was approved by the responsible ethics committee (Kantonale Ethikkommission Zürich, Ref. 2019–02002). Proximity extension assay (PEA) technology by OLINK was used for targeted proteomic serum analyses of 180 proteins measured in the OLINK immune response and inflammation panels (92 proteins each, 4 overlaps; Figure S1). Immune-inflammatory profiles of ice hockey players were compared at two time points (pre- and post-COVID-19). Additionally, post-COVID-19 profiles of ice hockey players were compared to the post-COVID-19 control group for reference. A detailed description of methods can be found in the Appendix S1.

Ice hockey players and control subjects reported comparable rates of previous SARS-CoV-2 infections, atopic comorbidities (asthma, allergic rhinitis), regularly occurring upper respiratory tract infections (URTI), fever, and recurrent herpes labialis (Table 1). COVID-19 vaccination history did not differ between athletes and controls. Control subjects reported a higher prevalence of symptoms in general and respiratory symptoms specifically. Clinical laboratory serum analyses showed no difference between ice hockey players and controls (Table S1).

PEA-based proteomic analyses of serum samples from elite ice hockey players identified 28 differentially expressed proteins involved in immune response and inflammation (Figure 1A,D) with a rather distinct representation of biological process networks (*t*-test results can be found in the Appendix S1). While similar process networks were found to be present at both sampling time points, specifically, lymphocyte proliferation (*CXCL12, CD40, PRKCQ, TNFSF14* pre-COVID-19 and *TRAF2, IRAK4, CASP-8* post-COVID-19) and innate inflammatory response (*PRKCQ, IRAK1*

Debbie J. Maurer and Elena Barletta equal first author contribution.