PROTOCOL

Creating A RIsk assessment biomarker tool to prevent Seasonal and Thunderstorm Asthma – CARISTA Study

Protocol Number 1
Version:2.0
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Authors:
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CONFIDENTIAL

Statement of Compliance

This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007, updated 2018), Australian Code for the Responsible Conduct of Research, 2018 *(the Code)* and the principles of the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

1. SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:			
Signature:		**Date:**	
Name (please print):	**Professor Jo Douglass**		
Position:	**Coordinating Principal Investigator**		

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2. CARISTA STUDY SYNOPSIS

Consumer Involvement	Confirm if there has been or will be consumer involvement and categorise as one of the following to align with OFR data collection requirements: • Consultative • Co-design • Nil consumer involvement Refer to section 12 for definitions and to provide details. This study was conceived following consultation with consumers' and professionals' organisations of Asthma Australia, the Australasian Society for Clinical Immunology and Allergy (ASCIA) and the Thoracic Society of Australia and New Zealand (TSANZ) who are partners in the grant for the development of the study protocol. A Consumer representative on the Study advisory panel will monitor and provide input into study progress and outcomes.
Design:	Phase 1: Prospective cohort study. Recruitment of a cohort of patients with seasonal allergic rhinitis (hay fever) with or without asthma, with prospective symptom monitoring for asthma and seasonal allergic rhinitis symptoms throughout two consecutive Melbourne springtime grass pollen seasons, with collection of physiological data at study entry together with storage of biological specimens for specified analysis at the completion of the recruitment.
Exclusion Criteria:	Individuals unable to provide informed consent or who do not meet inclusion criteria. Individuals who do not experience symptoms of seasonal allergic rhinitis. Individuals who do not consent to study procedures. People with pre-existing illness or medication use that would compromise participant safety or the derivation of biomarkers during the study.
Inclusion Criteria:	Consenting adults aged 18 to 70 years who have: • History of seasonal allergic rhinitis and/or are sensitised to ryegrass pollen (ryegrass pollen specific (RGP-sp) IgE >0.35kU/L. • Possess a smartphone or computer tablet or computer to participate in the prospective symptom data collection through a secure data platform. And who are willing to participate in: • Lung function testing • Blood sample collection for risk factor identification
Investigational product:	None
Lead Study Centres:	The University of Melbourne and The Royal Melbourne Hospital
Number of Planned Participants:	530

Primary Objectives:	To develop a biomarker tool to predict asthma exacerbations during springtime, in individuals who have seasonal allergic rhinitis through prospective symptom monitoring using a customised secure data platform over two consecutive springtime seasons.
Recruitment and data collection sites:	The Royal Melbourne Hospital (Melbourne Health) The Alfred Hospital (Alfred Health) The Northern Hospital (Northern Health) Sunshine and Footscray Hospitals (Western Health) Monash Medical Centre (Monash Health) The Austin Hospital Box Hill Hospital (Eastern Health)
Safety considerations:	This study is observational. No investigational product is being administered. Safety issues relate to a. Sample collection (blood, material for analysis) b. Responder burden of undertaking participation reporting hay fever/asthma symptoms
Secondary Objectives	To identify and validate biomarkers for asthma exacerbations in springtime including: a. Levels of sensitivity to rye-grass pollen and rye-grass pollen allergen components. b. Markers of cellular activation and inflammation. c. Lung function. d. To document the prevalence of severe and moderate asthma exacerbations in a cohort of ryegrass pollen sensitised patients over the springtime season.
Short Title:	CARISTA Study
Statistical Methods:	Descriptive and comparative statistics will be conducted on clinical and biological data. Logistic regression (binomial, multinomial and ordinal) analyses will be used to compare the clinical biomarkers with no asthma exacerbations, moderate and severe exacerbations. These models will include relevant confounders as identified through comprehensive causal diagrams using directed acyclic graphs. We will investigate selected variables for their potential to modify the relationship between the clinical biomarkers and asthma exacerbations. Statistical and machine learning methodologies will be applied to identify a potential model for the prediction of risk of asthma exacerbations based on the biomarkers. These models will be internally validated using cross-validation. Their predictive performances will be assessed with calibration, discriminative capacity and clinical utility via decision curve analysis. Furthermore, the relationships between daily allergic rhinitis and asthma symptoms with grass pollen levels and air quality will be examined using established case time series methodology which will control for confounding from air pollution and weather conditions.
Study Objectives:	1. To recruit a cohort of participants who have seasonal allergic rhinitis or asthma. 2. Obtain baseline demographic, historical health and allergic respiratory symptom severity and control data. 3. Obtain baseline blood and lung function samples for analysis.

	4. Obtain prospective springtime symptoms of asthma and seasonal allergic rhinitis along with pollen, weather and air quality data over two consecutive springtime seasons using a customised secure data platform. 5. To identify a treatable trait for seasonal allergic and thunderstorm asthma.
Study Question:	Can biomarkers of ryegrass pollen specific IgE, lung function and airway inflammation be used to identify patients with allergic rhinitis who are at greatest risk of allergic asthma exacerbation?
Subgroups:	
Title:	Creating A Risk assessment biomarker Tool to prevent Seasonal and Thunderstorm Asthma – CARISTA

3. Glossary of Abbreviations & Terms

Abbreviation	Description (using lay language)
FeNO	Fraction of Exhaled Nitric Oxide (ppb)
ACQ-5	Asthma Control Questionnaire- 5 item
SNOT-22	The Sino-Nasal Outcome Test
Lol p 1, Lol p 5	Allergen components of ryegrass pollen (*Lollum perenne*) of the major group 1 and 5 allergens.
PICF	Participant Information and Consent Form
RGP	Ryegrass pollen
RGP-A	Ryegrass pollen induced asthma
RGP-sp IgE	Ryegrass pollen specific immunoglobulin E
TA	Thunderstorm asthma
TNSS	Total Nasal Symptom Score
SAR	Seasonal allergic rhinitis

4. Study Sites

4.1 Principal Investigators and CHIEF & Investigators

Coordinating Principal Investigator		
Prof Jo Douglass	Director of Research and Professor of Medicine Royal Melbourne Hospital Department of Medicine, University of Melbourne T 03 8344 4578 E jdouglass@unimelb.edu.au jo.douglass@mh.org.au	Responsible for overall conduct of the study including ethics submission, data storage, analysis and financial accountability
Principal Investigators		
A/Prof Megan Rees	Head of Unit, Department of Respiratory and Sleep Disorders Medicine The Royal Melbourne Hospital – City Campus, Level 1, Centre for Medical Research Building 300 Grattan Street | Parkville Victoria 3050 E: Megan.Rees@mh.org.au	Respiratory clinician

Prof Christine McDonald	Director of the Department of Respiratory and Sleep Medicine, Austin Health E: Christine.mcdonald@austin.org.au	Respiratory and allergy clinician
Prof Mark Hew	Head of Allergy, Asthma & Clinical Immunology, Alfred Health Public Health & Preventive Medicine, Monash University E: M.Hew@alfred.org.au	Respiratory and allergy clinician
Prof Philip Bardin	Professor and Director Monash Medical Centre E: Philip.bardin@monash.edu	Respiratory and allergy clinician
Prof Frank Thien	Director of the Department of Respiratory and Sleep Medicine at Eastern Health, and the Monash University Eastern Health Clinical School. E: frank.thien@monash.edu	Respiratory and allergy clinician
A/Prof Anne Marie Southcott	Head of Unit Respiratory and Sleep Medicine Western Health E: AnneMarie.Southcott@wh.org.au	Respiratory and allergy clinician
Dr Katharine See	Director, Department of Respiratory Medicine The Northern Hospital E: Katharine.see@nh.org.au	Respiratory and allergy clinician
Chief Investigators		
Dr Rachel Tham	Department of Medicine, The University of Melbourne M: 0417 523 129 E: Rachel.Tham@unimelb.edu.au	Responsible for ethics submission, data storage, analysis and financial accountability, epidemiological and statistical aspects
Prof Janet Davies	Head of the Allergy Research Group, Centre for Immunology and Infection Control, QUT E: j36.davies@qut.edu.au	Responsible for molecular allergen characterisation.
Associate investigators		
Prof Adrian Lowe	Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics Melbourne School of Population and Global Health E: Lowea@unimelb.edu.au	Allergy epidemiologist
Prof Fay Johnston	Menzies Institute for Medical Research University of Tasmania; AirHealth Pty Ltd E: Fay.Johnston@utas.edu.au	Environmental epidemiologist and lead researcher of the AirRater prospective symptom monitoring app.
Dr Edwin Lampugnani	AirHealth Pty Ltd; School of Health Sciences, The University of Melbourne E: Ed@airhealthlab.com	Responsible for aerobiological aspects of the study
Dr Don Vicendese	Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics	Statistical analysis and mathematical modelling

	Melbourne School of Population and Global Health E: don.vicendese@unimelb.edu.au	
Prof Lena Sanci	Head of Department, Director of Teaching and Learning, and co-lead of the Children and Young People's Research Stream, Department of General Practice (DGP), University of Melbourne. E: l.sanci@unimelb.edu.au	Expert in co-design of interventions in general practice

4.2 HOSPITAL SITES

Site	Address	Contact Person	Phone	Email
The Royal Melbourne Hospital Dept of Respiratory and Sleep Medicine	300 Grattan St Parkville Vic 3050	Prof Jo Douglass A/Prof Megan Rees	(03) 9342 7191 (03) 9342 2880 (03) 9342 7191 (03) 9342 2880	jdouglass@unimelb.edu.au Jo.douglass@mh.org.au Megan.Rees@mh.org.au
The Austin Hospital	145 Studley Rd, Heidelberg VIC 3084	Prof Christine MacDonald A/Prof Christopher Worsnop	(03) 9496 3688	Christine.mcdonald@austin.org.au Christopher.worsnop@austin.org.au
The Northern Hospital	185 Cooper St, Epping VIC 3076	Dr Katharine See	0421 630 295	Katharine.see@nh.org.au
Western Health	Sunshine Hospital– 176 Furlong Rd, St Albans VIC 3021 Footscray Hospital - 160 Gordon St, Footscray VIC 3011	A/Prof Anne-Marie Southcott Dr Andrew Gillman	0407 339 127	AnneMarie.Southcott@wh.org.au andrewjg@hotmail.com
Monash Health, Monash	246 Clayton Rd, Clayton VIC 3168	Prof Philip Bardin Professor and Director	(03) 9594 2281; Fax (03) 9594 6415	philip.bardin@monash.edu

Medical Centre		Monash Lung & Sleep		
Alfred Health, Alfred Hospital		Prof Mark Hew A/Prof Joy Lee	(03) 9076 2934	M.Hew@alfred.org.au Joy.lee@alfred.org.au
Eastern Health, Box Hill Hospital		Prof Frank Thien	(03) 9095 2415	Frank.Thien@monash.edu
Non-Hospital Sites where participant data will be stored and analysed				
Queensland University of Technology		Prof Janet Davies	(07) 3138 3751	J36davies@qut.edu.au
The University of Melbourne		Dr Rachel Tham	0417 523 129	Rachel.Tham@unimelb.edu.au

4.3 PARTNER ORGANISATIONS

Organisation	Contact Person	Phone	Email
Asthma Australia	Anthony Flynn Senior Manager of Research, Information and Evaluation Laura Clarke Policy Advisor Asthma Australia	(03) 9086 7860	Aflynn@asthma.org.au lclarke@asthma.org.au
Australasian Society of Clinical Immunology and Allergy	Jill Smith ASCIA CEO		jill@allergy.org.au
The Thoracic Society of Australia and New Zealand	Dr Hayley See Research and Policy Manager	(02) 9222 6200	TSANZawards@thoracic.org.au
AirHealth Pty Ltd	Dr Edwin Lampugnani and Prof. Fay Johnston PO Box 38, Parkville, Vic 3052		theteam@airhealthlab.com

5. INTRODUCTION/BACKGROUND INFORMATION

5.1 LAY SUMMARY

Thunderstorm asthma is a recurring public health emergency in South-Eastern Australia with increasing risks due to climate change. Yet thunderstorm asthma is only the "tip of the iceberg" of documented seasonal surges in emergency asthma presentations due to ryegrass pollen allergy - an escalating challenge to healthcare provision in South-Eastern Australia. The CARISTA (Creating A Risk assessment biomarker tool to prevent Seasonal allergic and Thunderstorm Asthma) study aims to address these profound health impacts.
The CARISTA study will recruit adults at high risk of seasonal allergic asthma and thunderstorm asthma who will be monitored using a customised secure data platform for asthma and allergic rhinitis symptoms over two consecutive spring seasons (2025/2026/2027). Participants with self-reported or diagnosed seasonal allergic rhinitis and/or seasonal allergic asthma will be recruited before springtime and their baseline allergic rhinitis and/or asthma symptoms, respiratory function and blood biomarkers (allergy or sensitisation to ryegrass pollen, levels of inflammatory cells [eosinophils]) will be measured.

The key symptoms of interest are moderate or severe asthma exacerbations which are defined as an increase in asthma symptoms requiring intervention with an emergency medical visit, use of oral corticosteroid therapy or of regular preventive asthma therapy. These asthma exacerbations will be used to establish a biomarker-based estimate of risk for seasonal allergic asthma exacerbations to inform preventive clinical practice. The key biomarker will be the threshold of serum specific IgE (sp-IgE) to ryegrass pollen and its allergen sub-components, but other biomarkers such as lung function, eosinophil levels, and allergen component sensitisation will be simultaneously assessed.

This study brings together a team of world-leading *multidisciplinary* and *cross-sectoral clinicians and researchers* in Respiratory Medicine, Allergy, Primary Health Care, Epidemiology, Public Health, Statistical modelling and Botany from multiple health and academic institutions. Each clinician-investigator in this study is a member of the Victorian Department of Health Thunderstorm Asthma Advisory Committee and will bring CARISTA findings to the attention of governments and the communities at risk.

Overall, CARISTA will test a novel approach to predict risk for seasonal allergic asthma to identify treatable traits for preventing seasonal allergic asthma exacerbations to reduce the recurrent annual health threat of seasonal and thunderstorm asthma, address community and health care provider concerns and uncertainty regarding preventive treatment and management.

5.2 INTRODUCTION

On 21 November 2016, Melbourne suffered an asthma epidemic following a thunderstorm that overwhelmed emergency and health services and, tragically, 10 people died of asthma[1]. Only 43% of those who attended emergency departments had ever been diagnosed with asthma, but 87% had a history of hay fever (seasonal allergic rhinitis: SAR)[2]. This tragic event represents the 'tip of the iceberg' of Australia's allergic asthma burden. Each year there are springtime outbreaks of asthma emergency presentations throughout South-East Australia associated with prolonged high levels of airborne ryegrass pollen (RGP)[3]. The demands on health services highlight RGP-induced asthma as a major driver of asthma morbidity and mortality. If this remains unrecognised, and with predictions of increased allergenicity of pollens with climate change, it is inevitable that severe asthma epidemic events, such as occurred in 2016, will recur. The risks to individuals need to be quantified and preventive mitigation strategies are needed that are based on evidence that can apply at individual, clinician and public health levels. With approximately 19% of the Australian population reporting SAR[4], generalised prescription of preventive asthma therapy to every individual with SAR or RGP sensitisation is not a realistic preventive plan. Currently no biomarkers exist for the treatable trait of RGP-induced asthma or thunderstorm asthma in national or international guidelines.

The solution: Our study proposes to explore this problem by testing a novel and innovative approach to predicting risk for RGP-induced asthma and thunderstorm asthma by defining and quantifying existing (RGP-specific IgE, lung function, blood eosinophils) and novel (sp-IgE to RGP allergen components Lol p 1 and Lol p 5) biomarkers to identify the treatable traits for RGP-induced asthma and thunderstorm asthma exacerbation. We will generate evidence to support policy and public health recommendations and the implementation of our validated biomarkers into clinical practice to reduce individual and population health risks.
This study aims to address this by:

1. Recruiting patients in Victoria with seasonal allergic rhinitis who may or may not have suffered from asthma.
2. Collecting:
   i. Demographic, clinical, medical and medication history data
   ii. Self-reported data on severity of seasonal allergic rhinitis and asthma control
   iii. Lung function measures
   iv. Blood sample for standard allergic parameters: total IgE, specific IgE to RGP and its allergen components (Lol p 1 and Lol p 5).
3. To prospectively monitor participants throughout two consecutive (2025/2026/2027) springtime RGP seasons (October to December) for each participant using a customised secure data platform *("CARISTA symptom monitoring platform")* to record rhinitis and asthma symptoms and exacerbations.
4. To conduct prospective analyses to identify clinical, physiological and biological markers of asthma exacerbations.
5. To prospectively validate and identify environmental risk factors for allergic rhinitis and asthma symptoms in this cohort by linkage of symptom data gathered by the *CARISTA symptom monitoring platform* with meteorological, air quality and aerobiological data.

This study brings together a team of world-leading *multidisciplinary* and *cross-sectoral clinicians and researchers* in Respiratory Medicine, Allergy, Primary Health Care, Epidemiology, Public Health, Statistical modelling and Botany from multiple health and academic institutions. Our key consumer partner is the leading national consumer-led asthma advocacy organisation, Asthma Australia. Their research priorities are informed by in-depth consultation and national surveys of people living with asthma which have driven the conceptualisation of this study. The CARISTA research collaboration is underwritten by foundational partnerships with the professional associations in allergic and respiratory disease: the Australasian Society of Clinical Immunology and Allergy (ASCIA) and the Thoracic Society of Australian and New Zealand (TSANZ). These organisations engage directly with their members, key health professionals working with the populations at risk, and are responsible for developing and implementing *treatable trait*-based clinical guidelines. They will facilitate translation and implementation of CARISTA. Each principal clinician investigator in this study is a member of the Victorian Department of Health Thunderstorm Asthma Advisory Committee and will bring CARISTA findings to the attention of governments and the communities at risk.

The study is planned to begin prior to the 2025-2026 grass pollen season and recruit adults from September 2025 through to September 2026. Participants will form a cohort that will be prospectively monitored to validate findings found in prospective analyses. The project findings will be disseminated through scientific literature but will also be disseminated to the public, clinicians and policy makers through peak asthma bodies and clinical guidelines and public health initiatives.

5.3 BACKGROUND INFORMATION

On 21 November 2016, Melbourne experienced an extreme and unprecedented asthma epidemic following a thunderstorm that overwhelmed emergency services and led to the activation of disaster codes. Tragically, 10 people died of asthma in Melbourne due to this event. Health services were overwhelmed with 3365 excess emergency presentations, and a 10-fold increase in asthma related

hospital admissions occurred over the following day. Reports abounded of local medical practitioners and local pharmacies being swamped with requests for asthma treatment from people whose asthma had previously been mild, or even who were considered not to have asthma [5]. This event has provoked dynamic public health, emergency services and clinical responses based on previous evidence and expert opinion regarding the episode[6].

Clusters of epidemic thunderstorm asthma (TA) with marked increases in asthma hospital presentations for emergency care and asthma morbidity have been previously described in Melbourne and globally, In Melbourne, events have been described since 1984 with all events occurring in the month of November, and all have been associated with a thunderstorm during days of high grass pollen counts (Table 1). The storms have not uniformly been associated with rainfall. Retrospective examination of those who suffered in these epidemics suggested that a history of seasonal allergic rhinitis (SAR), with or without asthma symptoms, was universal. Furthermore, all had specific IgE to ryegrass pollen (RGP) which showed their sensitisation to RGP. Botanical studies revealed that RGP and Bermuda grass pollen has a propensity to "explode" into sub-fragments during conditions that involve rapid rises in humidity, leading to the induction of fine allergenic particles (2 to 5µm in diameter) which are respirable into the lower respiratory tract and can induce asthma symptoms.

Table 1: Summary of documented thunderstorm asthma epidemics in Melbourne

Date	Time of storm	Total hospitalised	Daily grass pollen levels (grains/m^3)			Rain (mm)
10/11/1984	7pm	Unknown	Unknown	Unknown	Unknown	12.4
8/11/1987	6pm	26	Unknown	Unknown	Unknown	45.6
29/11/1989	5pm	47	Unknown	Unknown	Unknown	7.8
19/11/2003	10pm	Same day: 27 Next day: 76	128	126	18	1.2
24/11/2010	10:30pm	Same day: 59 Next day: 144	56	104	117	23
21/11/2016	5:30pm	476	102	29	22	<1.0

Epidemic events of thunderstorm asthma have been described in New South Wales and internationally, with similar features, although none with the extent of mortality associated with the Melbourne 2016 event (Table 2).

Table 2

Country and year	Agent/Count	Health impact
Australia (Wagga Wagga, NSW), 30/10/1997 [7] **Previous less severe episodes: 1987, 1993, 1996**	Ryegrass pollen/count unknown	138 attended Wagga Wagga hospital
United Kingdom (London), 24/6/1994 [8,9]	Grass pollens/ 258 grains/m^3	640 attended hospitals, 104 were admitted including 5 into ICU.
USA[10]	*Alternaria* (mould/fungi) spores	
Canada[11]	Grass pollens	
Greece[12]	Grass and olive pollen	
Italy[13]	Olive Pollen	
Iran[14]	Not investigated	At least 2000 people attended ED

Australia, 21/11/2016	**Melbourne,**	Grass pollen >100 grains/m^3	3365 excess ED attendances; 476 hospitalisations; 35 admitted to ICU; 10 deaths

Examination of 20 years of hospital admission data has revealed that, state-wide high asthma admission days as defined by the numbers of asthma hospital presentations, exceeds seasonal averages by >4 standard deviations occur, with very few exceptions, in the 6-week period from the last week of October to the first week of December [3]. Within this period TA accounts for only some of these events. This suggests that in Melbourne at least, TA is the most severe of multiple events leading to asthma hospital presentations and admissions. This period correlates with seasonal allergies due to range of grass pollens, ambient fungal spores, high grass pollen aerobiological loads and meteorological conditions which produce submicronic particles of RGP. Consequently, this study proposal aims to focus on this time period from the final week of October to the last week of December.

Given the severity of the Melbourne 2016 event, public health and clinical recommendations have been made. The community has been advised to recognise that "Hay fever" is likely to be a risk factor for asthma during springtime and clinicians have been advised to provide action plans to all. However, the practicalities of this occurring are contested, given that as many as 1:6 suffer from SAR and most individuals self-medicate with treatment purchased from pharmacies. Moreover, in the asthma and allergy clinical communities controversy exists as to the role of short-acting β-2 agonists which can exacerbate airway hyper-responsiveness so that, whilst they do provide immediate symptomatic relief and were found to be extremely helpful to many, they were not preventive of emergency presentation in the only case-control study of TA[7]. Whilst most who were admitted to hospital had pre-existing asthma, other indicators for severity of TA attacks are not clear. Indicators of severity would enable targeting of resources to those most at risk, avoid the unnecessary prescription of corticosteroid medication and enable clinicians to strongly endorse asthma preventive treatments for those at great risk, a possibly life-saving treatment.

Therefore, questions arise regarding:

a) Identifying those most likely to suffer severe seasonal allergic asthma: Should all hay fever sufferers be encouraged to take preventive medications for asthma in addition to their hay fever treatment? Or can preventive asthma therapy and immunotherapy be reserved to reliably target those most at risk?
b) Do biomarkers exist for identification of severe asthma exacerbations including physiological data and demographic data?
c) The best preventive treatment for future events, including the role of preventive intranasal or inhaled corticosteroids and/or allergen immunotherapy.
d) Seasonal allergic asthma provides a unique opportunity to study allergic asthma. It is likely that biological studies will provide important discoveries regarding the genesis and persistence of allergic asthma and rhinitis which can inform the discovery of new therapies.

6. Study Objectives

6.1 Hypothesis

Primary hypothesis: A specific IgE to ryegrass pollen (RGP) above 10 kU/L predicts increased risk of asthma exacerbation during the RGP season.

Secondary hypothesis: RGP-specific IgE and sp-IgE to its allergen components are reliable and feasible quantitative biomarkers of the treatable trait for allergic asthma exacerbation risk.

6.2 AIMS

The CARISTA Study seeks to extend the retrospective observation from the Thunderstorm Asthma in Seasonal Allergic Rhinitis (TAISAR) study that allergic sensitisation, detected by allergen specific IgE to RGP, is an indicator of risk for TA.

The CARISTA Study is using a prospective cohort to undertake risk prediction modelling for RGP-asthma and TA and enable confidence in the translation of biomarkers to identify the treatable trait of seasonal allergic asthma.

STAGES

Stage 1: To establish a prospective cohort of individuals with seasonal allergic rhinitis in Melbourne with or without asthma.

This study aims to recruit adults who live in Melbourne in similar geographic areas to those who suffer from SAR, allergic asthma or thunderstorm asthma.
We will use multiple strategies to enhance our connections into the communities impacted by seasonal allergic asthma. Participants with SAR with/without asthma will be recruited through:

1. Study site coordinators will contact previous participants of other projects who have consented to be contacted about future research projects. These study site coordinators confirm the inclusion/exclusion criteria and provide written information and a consent form (printed version by mail or electronic pdf version by email) and arrange an appointment for them at one of the study sites when they are ready.
2. Recruitment posters will be displayed in the waiting areas of the participating hospital clinical sites;
3. Electronic advertisements/communications via websites and social media platforms of asthma and allergy support, advocacy and information services. For example, our partner organisations: Asthma Australia, the National Asthma Council, the Australasian Society for Allergy and Clinical Immunology and the Thoracic Society of Australia and New Zealand;
4. A pop-up notification to the subscribers of the AirRater and Melbourne Pollen apps who have consented to receiving these;
5. Recruitment posters in public areas.

It is planned to recruit 400 individuals who experience seasonal allergic rhinitis and are sensitised to ryegrass pollen for prospective monitoring of nasal, eye and lung symptoms during the spring season for two consecutive springtime seasons.

In order to recruit 400 individuals who are sensitised to ryegrass pollen we will need to recruit 530 individuals. Whilst those who are not sensitised to ryegrass pollen will not form part of intended primary analysis dataset we will ask non-sensitised to complete and report symptoms over the grass pollen season in order to study symptoms in non-sensitised participants.

Stage 2: Baseline data: demographic, clinical and physiological information.

At the hospital clinic the following information will be collected (See Attachment 9: CARISTA Participant Contact and ID Form & Attachment 1: CARISTA Baseline Data Collection Form):

1. Demographic data and information regarding a medical history of allergic diseases, asthma diagnosis, previous TA or RGP-asthma, medications for asthma and rhinitis and their doses, asthma exacerbation history, medical comorbidities, allergy status and other medications, contact details for GP.
2. History of requiring asthma hospital presentation, admission or intensive care presentation.
3. Current asthma control and hay fever severity
4. Lung function: Spirometry with bronchodilator reversibility.
5. Airway inflammation assessed using Fractional exhaled nitric oxide.
6. Blood for standard allergic parameters (total IgE, RGP-specific IgE, eosinophil count)
7. Blood (serum) for specific IgE to multiple allergen components related to hay fever and asthma especially Lol p 1, Lol p 5 and RGP-specific IgE.

Stage 3: To prospectively monitor allergic respiratory disease symptoms through two spring seasons between 2024-2026.

Participants who have a smartphone/computer tablet/computer will be requested to log their eye, nose and throat and lung symptoms, their severity, medication use and related medical attendance daily using the *CARISTA symptom monitoring platform* (Details are in Sections 6.3.2 and 7.4, See Attachment 11: CARISTA Symptom Monitoring Platform Workflow). Notifications to increase regular participation will be sent through the customised platform such as acknowledgement of their regular participation and symptom recording.

Participants without a smartphone/computer tablet/ computer will be contacted weekly by telephone to record their asthma and SAR symptoms, severity and medication use using the same questions as are in the *CARISTA symptom monitoring platform*.

Daily air quality, weather, air pollution and pollen count data will be provided by the AirHealth and will be available for subsequent geospatial analyses, but these environmental factors are not disclosed to participants through the *CARISTA symptom monitoring platform*.

This data collection will enable correlates of symptom severity with environmental conditions. It is planned that symptoms will be recorded daily for a maximum of 12 weeks during each springtime season. Participation in the symptom monitoring will be encouraged by automated nightly reminders at 8.00 pm if no data has been entered for that day. and by telephone follow-up by research staff for those who do not enter symptoms for a week during the study period.

The *CARISTA symptom monitoring platform* will provide overseeing clinical investigators with automated notifications of participants who have reported an increase in severity of asthma symptoms for two consecutive days or which require institution of preventer therapy or oral corticosteroid therapy. This will enable investigators at the enrolling site and the central (UoM/RMH) team to contact the study participant to verify the occurrence and severity of asthma exacerbations that occurred during the study period and advise if medical consultation is required. See Attachment 8: CARISTA Master Telephone Script.

Stage 4: To undertake statistical analysis and clinical risk prediction modelling.

Descriptive and comparative statistics will be conducted on clinical and biological data. Logistic regression (binomial, multinomial and ordinal) analyses will be used to compare the clinical biomarkers with no asthma exacerbations, moderate and severe exacerbations. We will investigate selected variables for their potential to modify the relationship between the clinical biomarkers and

asthma exacerbations. Multivariate analyses and machine learning methodology will be performed to identify a potential model for the biomarkers as a prediction of risk of asthma exacerbations.

6.3 OUTCOME MEASURES

6.3.1 CLINICAL, PHYSIOLOGICAL AND SYMPTOM VARIABLES

Clinical and physiological variables will be collected by questionnaire administered by the researchers (see Appendix 1). These include:

1. Demographic data and information regarding a medical history of allergic diseases, asthma diagnosis, previous TA or history of springtime allergic asthma (RGP-asthma), medications for asthma and allergic rhinitis and their doses, asthma exacerbation history, medical comorbidities, allergy status and other medications, contact details for general practitioner.
2. History (in the past 12 months or ever) of requiring asthma hospital presentation, admission or intensive care presentation.
3. Current (baseline) asthma control score (ACQ-5); and hay fever severity (TNSS) and Sino-Nasal Outcome Test (SNOT-22)
4. Lung function: Spirometry with bronchodilator reversibility. Some centres will have access to fractional exhaled Nitric Oxide measurements (FeNO). Sites who choose to perform this will record this measure.

Samples for laboratory analysis

Blood (25mls) will be collected to enable exploration of biological endpoints as markers of propensity and risk. Specific samples to be collected include:

1. Blood for full-blood examination, specifically eosinophil count (5.0mls, EDTA tube)
2. Blood for standard allergic parameters (total IgE, RGP specific IgE) (10.0 mls, serum tube)
3. Blood for specific IgE to allergen components especially RGP, Lol p 1 and Lol p 5 using allergens (10.0 mls serum tube)

6.3.2 PROSPECTIVE MONITORING OF SYMPTOMS

The **primary outcome measure** is the occurrence of severe and/or moderate asthma exacerbations occurring in each participant during the defined grass pollen season in each observation year. **Asthma exacerbation** is indicated by an increase in asthma symptoms requiring institution of preventer asthma therapy, asthma symptoms requiring oral corticosteroid therapy, institution of emergency asthma treatment or medical attendance for emergency asthma treatment indicative of uncontrolled asthma.

A customised secure data platform will be configured using the University of Melbourne REDCap database to enable study participants to prospectively record their hay fever and asthma symptoms through their smartphone/computer tablet/or computer for the 2025/2026/2027 springtime grass pollen seasons.
The flow chart of questions and range of possible responses are detailed in Attachment 11: CARISTA Symptom Monitoring Platform Workflow.
Participants will be informed at the time of recruitment, obtaining consent and every time they submit a report that their responses are not monitored in real-time or after-hours.
They will be requested to log their symptoms, medication use and any health care utilization, and their general location on a daily basis throughout the springtime grass pollen season: First week of October

to end of December. The *CARISTA symptom monitoring platform* will prompt each participant to enter a symptom log if no report is received by 8.00pm daily.

The *CARISTA symptom monitoring platform* will gather:

a) Daily symptom monitoring
    i. Total Nasal Symptom Score (TNSS – Attachment 4)
    ii. Lungs: wheeze, cough or shortness of breath; severity rated mild, moderate and severe
    iii. Eyes: itchy, watery symptoms present; severity rated mild, moderate and severe
    iv. Other: as described by participant in a self-completed text field
b) Daily medication use
    i. Asthma preventer: type nominated by study participant;
    ii. Asthma reliever: type nominated by study participant;
    iii. Asthma Maintenance And Reliever Therapy (MART linked by analysis of (i) and (ii) fields)
    iv. Oral corticosteroid: for respiratory symptoms, type nominated by study participant
    v. Antihistamines (oral): participant to record brand at enrolment, revised for year 2 or when needed through season
    vi. Steroid nasal spray: participant to record use and brand at enrolment, revised for year 2 or when needed through season
    vii. Topical ocular therapy (eye drops): participant to record brand at enrolment, revised for year 2 or when needed through season
    viii. Other: as described by participant in a self-completed text field; to record brand at enrolment, and can revised for year 2 or when needed through season
c) Any healthcare utilisation to manage or treat asthma symptoms.
d) Self-report of their location at State/Territory level, then if in Victoria, at metropolitan suburb/regional town levels.

6.3.3 INFORMATION PROVIDED BY THE AIRHEALTH ENVIRONMENTAL MONITORING SERVICE

AirHealth will provide the following data:

a) Daily Pollen Count captured from the closest pollen monitoring location at the time of symptom logging during the pollen season (there are 8 pollen monitoring stations in Victoria: Parkville, Burwood, Geelong, Creswick, Bendigo, Churchill, Dookie, Hamilton, Albury-Wodonga)
b) Measurement details of potential environmental triggers from the nearest environmental monitoring station at the time of symptom logging during the pollen season
    a. Air quality: particulate matter ($PM_{2.5}$ and PM_{10}), ozone [24-hour average, maximum]
    b. Meteorological (weather) conditions: ambient temperature (minimum, maximum, average), humidity, rainfall [24-hour average]

The *CARISTA symptom monitoring platform* will be designed for use on smartphones/computer tablets or computers and so will not be able to be completed by participants without any of these devices. Participants who do not have a smartphone/computer tablet or a computer for this component of the study will be unable participate in the daily prospective symptom monitoring, but will be contacted weekly to report their asthma control, allergic rhinitis symptoms and medications retrospectively using the same questionnaire.

7. STUDY DESIGN

7.1 STUDY TYPE & DESIGN & SCHEDULE

This prospective cohort study aims to recruit a cohort of participants who suffer from seasonal allergic rhinitis for prospective monitoring of allergic respiratory symptoms over two springtime seasons. Participants to be recruited are consenting adults aged ≥ 18 - 70 years reporting a history of seasonal allergic rhinitis and/or who are positive on blood specific IgE testing to RGP (RGP-sp IgE >0.35 kAU/L) and who are willing to participate in

a) Lung function testing
b) Blood sample collection for risk factor identification, and
c) Prospectively logging symptoms and medications throughout the springtime season using the digital platform or consent to a weekly phone call (in their preferred language) for asthma control, allergic rhinitis symptom and medication use data collection.

Participants will be recruited through participating Victorian hospital clinical sites, from communications through asthma and allergy websites through our partner organisations and the AirHealth suite of services including the AirRater and Melbourne Pollen Apps. We aim to recruit a cohort of up to 530 individuals during 2025 and 2026 and 2027, of whom, at least 400 should be sensitised to RGP.
We plan to undertake baseline data collection and activate prospective symptom monitoring in October 2025 and October 2026 and 2027. Our calculations indicate that 530 individuals will need to be recruited to enable 400 to complete.

Data to be collected, generated and stored:

1. All participants will be identified by a unique Participant ID comprised of:
   - CARISTA study - CSTA
   - Assessment site code (i.e. hospital code: Royal Melbourne Hospital = RMH; Northern Hospital = NTH; Western Health = WTH; Box Hill Hospital = BXH; Alfred Hospital = ALF; Monash Health = MON; Austin Health = AUS)
   - Hospital record number when available or when not, a study code e.g. FGH0099900
   - Participant's initials of first and last names
     - For example: CSTARMH0123456TR
2. Each Participant ID will be assigned a linking Study Code (See Attachment 9: CARISTA Participant Contact and ID Form) that will be used in the study's databases. For example, Unique ID = CSTARMH0123456TR, Study Code = CSTA0001

3. Each unique Participant ID linked to their Study Code will be stored in an approved secure REDCap database *["CARISTA ID linkage database"]* at the University of Melbourne (e.g. STARMH0123456TR, Study code = CSTA0001) accessible only to CIA Douglass and the Study Coordinator. Only study codes will be used to identify participant study data. This identification will enable coding for purposes of the study and linking of clinical and demographic data with data reported in the *CARISTA symptom monitoring platform*.

4. Identifying information
   - Signed Consent Form – paper and scanned copies
   - Identifying information: name, date of birth, address, hospital record number (if available), contact phone number, contact email.
   - Contact details of their medical practitioner

- Paper based consent forms will be stored in locked filing cabinets in the office of CI Douglass at the Department of Medicine, Royal Melbourne Hospital, The University of Melbourne
- Scanned and electronic data containing identifying information will be uploaded into the *CARISTA ID linkage database as* per point 3 above.

5. Demographic and clinical data collected at the initial clinical visit will be identified using the participant's study code (see Attachment 9: CARISTA Participant Contact and ID Form) and entered directly into a REDCap study database *["CARISTA demographic and clinical database"]* and kept under password control on a secure server at the Department of Medicine at the University of Melbourne. This database is accessible to only CI Douglass, the Study Coordinator and data analysts as approved by the CARISTA Executive Committee.
6. Demographic and clinical data collected will be according to the CARISTA Baseline Data Collection Form (Attachment 1). These include:
   - A questionnaire relating to respiratory allergic health validated through use in the TAISAR study (ref 14)
   - an Asthma Control Questionnaire (ACQ-5 – Attachment 2)
   - a Total Nasal Symptom Score (TNSS) and Sino-Nasal Outcome Test (SNOT-22 – Attachment 3) questionnaire to measure allergic rhinitis severity
   - Physical examination including height, weight, heart rate, blood pressure.
   - Physiological lung function data will be collected using a standard spirometer calibrated and operated to ATS/ERS criteria using the Global lung function initiative (GLI) predicted equations. Post-bronchodilator spirometry will be collected 15 minutes following the inhalation of 400-800mcg of inhaled salbutamol. Results will be printed and recorded on the data collection sheets. In study laboratories with capacity, there will be measurement and recording of fractional exhaled nitric oxide (FeNO).

Spirometry is the standard test for asthma and is a test of maximal expiration. Maximal expiratory efforts are made on several occasions to obtain a reproducible maximal effort. A standard dose of short-acting beta-2 agonist is then administered and after a wait of 15 minutes a repeat measure of spirometry is performed. This is a routine procedure for measurement of lung function and is safe with very few side effects.

Blood (25mls, approximately 1½ tablespoons) will be drawn for collection of serum for allergic antibody tests and blood examination.

Study participants will be trained in person on how to access the *CARISTA symptom monitoring platform*. They will need to know how to do this using the text message and/or email link which will be sent to them during the study period/s. The *CARISTA symptom monitoring platform* enables participants to provide their general location by inputting a postcode/suburb and then record and rate (mild, moderate, severe) their daily lung symptoms (wheezing, shortness of breath, chest tightness), nasal symptoms (runny, itchy, blocked) and eye symptoms (itchy, runny) as well as any medications they have taken to manage these symptoms and whether they have sought health care advice/treatment. Participants are requested to record daily symptoms on the *CARISTA symptom monitoring platform* from the beginning of October to end of December. Data collected is matched by their general location to the ambient pollen levels, weather conditions and air quality data recorded at the nearest monitoring station/s (provided by AirHealth). Data is stored on a secure REDCap server at the University of Melbourne.

Participants will be reimbursed for car parking or public transport expenses for their research project visit for clinical data collection. This is limited to a maximum of $55 per participant.

Study procedures will involve a single visit which is likely to take 2 hours. It will involve:

Assessment/Procedure	**Visit 1**
Informed Consent	✓
Collection of Demographic Information	✓
Height, Weight Measurement	✓
Heart rate and blood pressure	✓
Lung Function and airway inflammation (Spirometry, FeNO)	✓
Questionnaires (Respiratory allergy questionnaire, medications, ACQ-5, SNOT-22, TNSS)	✓
Blood Collection (25mls)	✓
CARISTA symptom monitoring platform training	✓

7.2 Standard Care and Additional to Standard Care Procedures

This is an observational study. All participants will have procedures that, whilst part of standard allergy and respiratory care and of negligible clinical risk, may not be part of this individual's care unless they have been referred for specialist management for their seasonal symptoms of allergic rhinitis or seasonal allergic asthma. Where this information has been gathered in the preceding 6 months, it will be taken from existing clinical records where available rather than being repeated for this study unless new treatment involving oral steroids, inhaled corticosteroids or biologics has been instigated or the treatment regimen has been changed since the historic readings. Since many potential participants for this study will be seeking care for seasonal allergic rhinitis or asthma symptoms, the study may overlap with an assessment of allergic disease.

Standard Procedures:

- Collection of demographic and historical clinical data
- Physical examination including heart rate, blood pressure.
- Spirometry and fractional exhaled nitric oxide (FeNO)
- Blood collection for
  - Total IgE
  - Serum-specific IgE to ryegrass pollen and ryegrass pollen components Lol p 1 and Lol p5
  - Full Blood Examination

Additional to standard care:

- Participation in the *CARISTA symptom monitoring platform* is not part of standard care.

7.3 Study Methodology

This is a prospective observational cohort study.
Individuals who have seasonal allergic rhinitis will be invited to participate.
Once they agree they will be seen by a study nurse or an investigator who will obtain informed consent and then undertake the following:

- Collection of demographic and historical clinical data

- Physical examination including height, weight, heart rate, blood pressure
- Measurement of lung function using spirometry: a standard measure of respiratory function performed by exhalation into a spirometer.
- Measurement of airway inflammation using FeNO
- Blood collection (25mls) for Melbourne Health Pathology
    - Total IgE and serum-specific IgE to ryegrass pollen (RGP)
    - Full blood analysis (FBE)

    - Samples for refined serological investigations for multiple allergen components sensitivity will be transferred to an external laboratory with the requisite expertise and resources at Queensland University of Technology, led by CI Prof Janet Davies
        - An array of hay fever and asthma related environmental allergens, but especially to specific IgE to RGP, Lol p 1 and Lol p 5 antigens

On the day the study participants attend for the testing, they will be asked that they

a) Do not take asthma reliever medication for at least 4 hours before the tests. If they have severe or mild asthma on the day, they can call and talk with the study nurse as participants would not be asked to refrain from using their asthma medication as it could put them at risk of experiencing severe asthma.

7.4 CARISTA SYMPTOM MONITORING PLATFORM

It is requested that study participants enter their symptoms each day during the study from the first week of October until the end of December. This takes about 1 to 5 minutes depending on the number of symptoms they are reporting. Participants will be informed at the time of recruitment, obtaining consent and every time they submit a report that their responses are not monitored in real-time or after-hours.

The flow chart of questions and range of possible responses are detailed in Attachment 11: CARISTA Symptom Monitoring Platform Workflow.

The CARISTA symptom monitoring platform is located on a REDCap database within the secure research environment at the University of Melbourne. The process involved in submitting symptom information into the *CARISTA symptom monitoring platform* via smartphone/computer tablet/computer using a text message or email link will be explained by the study nurse at enrolment.

At the clinical data collection session, a series of questions will obtain their history of asthma and/or hay fever. Medications used for allergic rhinitis and asthma will be entered. This will enable calculation of asthma and rhinitis symptoms scores, and a combined rhinitis and medications score.

To verify that the correct person is completing the questionnaire, we will request the respondent to provide their first name and date of birth at baseline entry. If there is discrepancy between the information provided by the respondent and the details recorded in their record, then the study team will contact the participant by phone or email to resolve the discrepancy. If the data does not belong to the correct CARISTA participant, it will be removed from the *CARISTA symptom monitoring platform.*

During symptom monitoring, the *CARISTA symptom monitoring platform* will request the participants to enter their suburb/postcode as their location when symptoms are logged so that the measures from the nearest air quality, weather and pollen monitoring stations are extracted and added to the

symptom log record. Participation in the daily symptom monitoring will be encouraged by automated nightly reminders at 8.00 pm if no data has been entered for that day. and by telephone follow-up by research staff for those who do not enter symptoms for a week during the study period.

Data collected by the digital platform will be securely stored on approved University of Melbourne REDCap database servers accessible to only CI Douglass and the Study Coordinator.

7.5 Study Extension

We intend to undertake a qualitative study using co-design methods to plan implementation and translation of the study findings. This is additional to this study and will be submitted in a subsequent ethics application. This study will ensure patient and clinician engagement in strategies for implementing study findings.

8. Study Population

8.1 Recruitment Procedure

This study aims to recruit adults who live in Melbourne in similar geographic areas to those who suffer from seasonal allergic rhinitis (SAR), allergic asthma or thunderstorm asthma.

We will use multiple strategies to enhance our connections into the communities impacted by seasonal allergic asthma. Participants with SAR with/without asthma will be recruited through:

1. Study site coordinators will contact previous participants of other projects who have consented to be contacted about future research projects. These study site coordinators confirm the inclusion/exclusion criteria and provide written information and a consent form (printed version by mail or electronic pdf version by email) and arrange an appointment for them at one of the study sites when they are ready. See Attachment 8: CARISTA Master Telephone Script
2. Recruitment posters will be displayed in the waiting areas of the participating hospital clinical sites; See Attachment 5: CARISTA Master Recruitment Flyer.
3. Electronic advertisements/communications via websites and social media platforms of asthma and allergy support, advocacy and information services. For example, our partner organisations: Asthma Australia, the National Asthma Council, the Australasian Society for Allergy and Clinical Immunology and the Thoracic Society of Australia and New Zealand; See Attachment 6: CARISTA Advertising text.
4. A pop-up notification to the subscribers of the AirRater and Melbourne Pollen apps who have consented to receiving these; See Attachment 7 CARISTA Advertising Text.
5. Recruitment posters in public areas. See Attachment 5: CARISTA Master Recruitment Flyer.

The ethics-approved recruitment notices will contain links (URL, QR code) to the CARISTA Study website that will be hosted on the University of Melbourne secure data servers. This website will describe the background, aims and purpose of the CARISTA Study and a description of the types of participants needed for the study. There will be a link embedded in the site which will take potential participants to a secure REDCap questionnaire which forms the *"CARISTA EOI database"* which will step the prospective participants through the exclusion/inclusion criteria. If they meet the inclusion criteria then they will proceed to a page where they can register their interest to participate in the CARISTA Study. They will provide their name, contact details (email or phone), age and gender and they will be informed that a study coordinator will be in contact with them to explain the project in more detail, answer any questions and inform them that they are under no obligation to participate and may withdraw before the study is complete. See Attachment 7: CARISTA Website and Participant Expression of Interest text.

The CARISTA study coordinator will contact participants who indicate interest in participating in the study via the CARISTA website and confirm the inclusion/exclusion criteria and provide written information and a consent form (printed version by mail or electronic pdf version by email) and arrange an appointment for them at one of the study sites when they are ready. See Attachment 8: CARISTA Master Telephone Script and Attachment 9: CARISTA Participant Contact and ID Form

It is planned to recruit 400 individuals who experience seasonal allergic rhinitis and are sensitised to ryegrass pollen (RGP) for prospective monitoring of nasal and lung symptoms during the spring season for two consecutive springtime seasons. In order to recruit 400 individuals who are sensitised to RGP we will need to recruit 530 individuals. Whilst those who are not sensitised to RGP will not form part of intended primary analysis dataset we will ask non-sensitised to complete and report symptoms over the grass pollen season in order to study symptoms in non-sensitised participants.

8.2 INCLUSION CRITERIA

Consenting adults aged ≥ 18 - 70 years reporting a history of seasonal allergic rhinitis. The primary analysis dataset will be in those who are positive on blood specific IgE testing to RGP (RGP sp IgE ≥ 0.35 kAU/L). However, individuals who are not sensitised to RGP (i.e. blood RGP sp-IgE < 0.35 kAU/L) will be prospectively monitored throughout the pollen season to provide a control dataset.
And who are willing to participate in

a) Lung function testing
b) Blood sample collection for risk factor identification, and
c) Prospectively logging their symptoms and medications through the springtime season using the *CARISTA symptom monitoring platform* or consent to a weekly phone call (in their preferred language) for symptom data collection.

8.3 EXCLUSION CRITERIA

a) Children <17 years of age
b) Individuals unable to provide informed consent
c) Individuals who do not suffer from symptoms of seasonal allergic rhinitis
d) Individuals who do not consent to lung function testing and blood sample collection
e) Individuals with unstable asthma (FEV1 by spirometry less than 70% predicted), a recent exacerbation or change of asthma preventive medication use (within one month) would be excluded, although re-screening would be permitted after one month, time permitting.
f) Individuals with severe asthma requiring the use of continuous oral corticosteroids or biological medication for severe asthma.
g) The presence of any medical illness, such as cardiac disease, pre-existing illness or immunomodulatory therapy that, in the opinion of the Investigators, would compromise participant safety or the derivation of biomarkers during the study.

8.4 CONSENT

Participants will provide written, informed consent. Study nurses, coordinators, clinicians and investigators are qualified to obtain informed consent for this study.
Potential participants whose first language is not English but who have indicated a willingness to undertake the study will be recruited with the aid of phone interpreters paid for by the study.
Participants will asked for their consent if they wish to be contacted to receive a summary of the study's findings.
Participants will be informed of potential future use of the de-identified study data:

- Provision to journal or online repository, if required;
- Provision to other research groups undertaking secondary analyses (e.g. systematic reviews, meta-analyses);
- Future grant applications;
- Future research projects that are an extension or closely related to this study, subject to appropriate review and approval by a relevant human research ethics committee

Under these conditions when this study's data may be used, it may not be appropriate or practical to contact all participants. Hence, they will be asked for their permission to reuse the data for any of these potential uses without contacting them. In addition, for other future research, they will be asked if the study team can contact them to invite them to participate in any future studies. The options are to be selected on the Consent Form.

9. Participant Safety and Withdrawal

9.1 Risk Management and Safety

a) Questionnaires and symptom collection

Collection of patient data by questionnaire can occasionally be distressing for participants who have suffered significantly through asthma, TA or seasonal allergic rhinitis. In these instances where further care is required the individuals will be referred back to the principal investigator or primary clinician at their study site. The participant's local medical/general practitioner (GP) will also be advised of this issue subject to the participant consenting to this.

b) Lung function testing

Lung function testing (spirometry) is a safe procedure routinely conducted to assess asthma and related respiratory diseases. The risk from this is minimal, although spirometry is a test of maximal respiratory effort, so some short-lived fatigue is common. Participants will be advised of this during the consent process.
Standard safety procedures include conducting testing by trained study personnel and following standard protocols for spirometry as recommended by the internationally recognised ATS/ERS guidelines for lung function testing. (ATS/ERS taskforce: Standardisation of Lung Function testing. Eur Respir J 2005; 26: 319–338). Exhaled Nitric Oxide testing is an expiratory test of submaximal effort and therefore has minimal side effects.

c) Blood sampling

This study requires 25mls of blood for processing. Blood sampling will be performed by venipuncture of the antecubital vein by qualified research staff. This procedure is slightly painful, and the major complication is bruising at the site of venipuncture. This recovers within a few days. Participants will be advised of this during the consent process.

d) Unexpected abnormal results

Unexpectedly abnormal results, such as significantly abnormal lung function or blood tests will be reported to the principal investigator/s at each study site and a request for follow-up made. Investigators at each site, or failing that the principal clinical investigators will determine the best course of action. This may be to repeat the lung function and/or blood tests (at no cost to the participant), a referral to a specialist clinic or discussion with the participant as to the best course of action. Their GP will be informed of all results should the participant consent to this.

e) Compromise of confidentiality and privacy of information

Participants' identifying data will be assigned a unique Participant ID and coded with a unique Study Code and will be removed and stored in a University of Melbourne REDCap database separately from any clinical, demographic and symptom data. This dataset will be secured and settings will only allow access by CI Douglass and the Study Coordinator.
The clinical, demographic and symptom data of each participant will be assigned the unique Study Code and stored in a University of Melbourne REDCap database separately from any identifying information. This dataset will be secured and settings will only allow access by CI Douglass and the Study Coordinator and CARISTA Executive Committee approved data analysts.
All paper-based information will be securely kept in locked cabinets in a locked office in the Department of Medicine (University of Melbourne, Royal Melbourne Hospital) until electronically archived 15 years after study completion. At that time, paper-based records will be securely destroyed using University of Melbourne document destruction.
Computer records will be kept on secure networks under password control accessible only to investigators at each study site.
Electronic records will be archived 15 years after study completion according to institutional guidelines applying at that time.

Any adverse events associated with the study will be documented (see Attachment 12: CARISTA Asthma Outcomes and Adverse events Source Document) and followed up in entirety by the clinical research team in consultation with the Study Safety and Outcome Validation Committee (see Section 13 Study Governance and Management). The data will be entered into the REDCap *"CARISTA Asthma Outcomes and Adverse Events database"*

9.2 HANDLING OF WITHDRAWALS

Participants may withdraw from the study at any time by notifying investigators of their wish to do so. They may formally document this by signing a withdrawal of consent form (See Attachment 10: CARISTA Master Main PICF & Withdrawal of Consent Form, Version 2.0, Page 13).

If this occurs no further information will be collected, but personal information already collected will be retained to ensure that the results of the research project are measured properly in compliance with legal requirements. Data collected up to the time of withdrawal will form part of the research results.

Participants will be advised of this during the consent process.

9.3 REPLACEMENTS

Not applicable

10. STATISTICAL METHODS

10.1 SAMPLE SIZE ESTIMATION & POWER CALCULATIONS

In our pilot TAISAR study 78% (176/226) individuals recruited with SAR had RGP-sp IgE levels > 0.35 kAU/L [15]. Using this prevalence assumption and binomial distribution we calculated that to have an 80 - 90% probability of finding at least 400 participants with RGP-sp IgE of >0.35 kAU/L, 524-529 eligible participants would need to be screened for the CARISTA study. We will screen 530 individuals to recruit the 400 participants needed to validate the RGP-sp IgE biomarker in a clinical risk prediction model for RGP-A and TA exacerbations. The sample size for the clinical risk prediction model was calculated using the TAISAR data from a range of clinical tests to predict TA hospitalisation [15] and the following sample size model [16]. Using the assumptions that the clinical biomarkers AUC_{ROC} ranging from

0.57-0.74, the prevalence of TA symptoms was between 0.28-0.37 and the number of parameters to be included is 10, the minimum sample size required for new model development of the biomarkers of interest is 344. The proposed sample size of 400 enables the clinical risk prediction model to have power to ensure the margin of error is ~0.05, the mean error across participants is <10% and includes a shrinkage factor of 10% to guard against overfitting. These power calculations were provided by co-investigators Dr Rachel Tham and Professor Adrian Lowe, both experts in biostatistics and epidemiology.

10.2 STATISTICAL METHODS TO BE UNDERTAKEN

Descriptive and comparative statistics will be conducted on clinical and biological data.
Logistic regression (binomial, multinomial and ordinal) analyses will be used to compare the clinical biomarkers with no asthma exacerbations, moderate and severe exacerbations. These models will include relevant confounders as identified through comprehensive causal diagrams using directed acyclic graphs. We will investigate selected variables for their potential to modify the relationship between the clinical biomarkers and asthma exacerbations.
CI Dr Vicendese, a mathematical biostatistician, will conduct multivariate analyses to develop a statistical model for the prediction of risk of asthma exacerbation based on the biomarkers. Further models will be developed using machine learning (ML) methodology (e.g. random forest or neural nets, which accommodate complex interactions and collinearity between biomarkers, individual characteristics and exposures). The statistical and ML models will be internally validated using a cross-validation scheme. Their predictive performances will be assessed and compared based on calibration, discriminatory ability (i.e. AUC_{ROC}) and clinical utility via decision curve analysis.
We will use the TRIPOD checklist for prediction model development [17]. This comprehensive process will determine how risk of exacerbation varies with biomarker levels and enable precision in understanding their impacts via the identified treatable traits.
The relationships between daily allergic rhinitis and asthma symptoms with grass pollen levels will be examined using established case time series methodology [18] which will control for confounding from air pollution and weather conditions.

11. STORAGE OF BLOOD AND TISSUE SAMPLES

11.1 DETAILS OF WHERE SAMPLES WILL BE STORED, AND THE TYPE OF CONSENT FOR FUTURE USE OF SAMPLES

Participants will be asked to consent for serological testing and full blood examination.
Blood samples will be labelled with Study Codes and transported to the Royal Melbourne Hospital Department of Pathology for processing.

Serum samples for transfer to PI Prof Janet Davies will be labelled with Study Codes, frozen and stored at -70C at the Royal Melbourne Hospital Department of Pathology until recruitment is completed. The coded samples will then be transferred to the Queensland University of Technology (PI Prof Janet Davies) for a wide array of environmental allergen components analyses, especially RGP, Lol p 1 and Lol p 5.

Blood results from the full blood examination (eosinophil count), total IgE and ryegrass pollen- specific IgE pollen, all routine medical laboratory, NATA-accredited tests, will be entered into the *"CARISTA demographic and clinical database"* and will be copied to the participant's family doctor (GP) and hospital medical record to enable incorporation into the participant's clinical record.

Tests for allergen components sensitivity (especially, specific IgE to Lol p 5 and Lol p 1) are not routine clinical tests and will not be released to individuals or their GP. Following the study's completion the

predictive value of these tests may be established, and results may then be reported in follow-up communications to participants and their health-care providers if their predictors are positive.

12. Data Management, Security & Handling

12.1 Details of where records will be kept & How long will they be stored

Study records will be maintained at the Department of Medicine, University of Melbourne (Royal Melbourne Hospital).
Each individual study site that collects the data will have the original documents including:

- Participant Information and Consent Form (PICF)
- Spirometry and FeNO results
- Copies of pathology analysis results.

Original documents will be scanned and both original and scanned copies will be forwarded to the Department of Department of Medicine, University of Melbourne, Royal Melbourne Hospital where they will be filed on the secure research management database within the University of Melbourne. Following completion of the study and anticipated initial analysis and publication of the first paper (2030), paper records will be destroyed. The electronic records including scanned consent and questionnaire forms will be maintained as recommended.

Data will be kept at each participating institution for 15 years. After this time the original documents will be securely destroyed.

Each study site will assign participants a unique study code consisting of:

- CARISTA study - CSTA
- Assessment site code (i.e. hospital code: Royal Melbourne Hospital = RMH; Northern Hospital = NTH; Western Health = WTH; Box Hill Hospital = BXH; Alfred Hospital = ALF; Monash Health = MON; Austin Health = AUS)
- Hospital record number when available or when not, a study code e.g. FGH0099900
- Unique ID for each participant which is stored in a separate linkage database
- For example, Unique ID = CSTARMH0123456TR, Study Code = CSTA0001

A password protected file of participant identifiers (Name, DOB, Hospital record number, contact phone number, address, email address) linking patient identifying and contacting data with study codes will be kept separately from clinical data and thereafter all participant data will be referenced by study code. The file linking study codes with patient identifiers will be kept at the Department of Medicine, University of Melbourne in a secure password protected folder on a secure network with access limited to the Principal Investigators and the study personnel required to enter the data. This data will be kept for 15 years following study completion and then securely archived or destroyed as per established institutional guidelines and protocols.

All participant data will be captured and stored electronically on secured REDCap database servers at the University of Melbourne which are password protected. Upon receipt of participant data at the University of Melbourne, data will be de-identified and files linking participant identification will be separated from all study data. This data will be kept on a secure network and database within the secure research environment of the University of Melbourne with password protection and accessible only to the Principal Investigators.

12.2 Confidentiality and Security

All participants will be identified by a unique Participant ID comprised of:

- CARISTA study - CSTA
- Assessment site code (i.e. hospital code)
- Hospital record number when available or when not, a study code e.g. FGH0099900
- Participant's initials of first and last names
  - For example: CSTARMH0123456TR

Each Participant ID will be assigned a linking Study Code that will be used in the study's databases. For example, Unique ID = CSTARMH0123456TR, Study Code = CSTA0001

Each unique Participant ID linked to their Study Code will be stored in an approved secure University of Melbourne REDCap database *"CARISTA ID linkage database"* at the University of Melbourne (e.g. STARMH0123456TR, Study code = CSTA0001) accessible only to CIA Douglass and the Study Coordinator. Only study codes will be used to identify participant study data. This identification will enable coding for purposes of the study and linking of clinical and demographic data with data reported in the *CARISTA symptom monitoring platform*.

Identifying information consist of:

- Signed Consent Form – paper and scanned copies
- Identifying information: name, date of birth, address, hospital record number (if available), contact phone number, contact email.
- Contact details of their medical/general practitioner

Paper based consent forms will be stored in locked filing cabinets in the office of CI Douglass at the Department of Medicine, Royal Melbourne Hospital, The University of Melbourne
Scanned and electronic data containing identifying information will be uploaded into the *"CARISTA ID linkage database"* as per point 3 above.

Demographic and clinical data collected at the initial clinical visit will be identified using the participant's Study Code and entered directly into a University of Melbourne REDCap study database, the *"CARISTA demographic and clinical database"*. Seasonal symptom reports will be identified using the participant's Study Code and entered directly into a University of Melbourne REDCap study database, the *"CARISTA Symptom Monitoring Database"*. These databases will kept under password control on a secure server at the Department of Medicine at the University of Melbourne. This database is accessible to only CI Douglass, the Study Coordinator and data analysts as approved by the CARISTA Executive Committee. An extract of de-identified, non re-identifiable study data for example demographics; year of birth, gender, will be shared with PI Davies to enable assay development for spIgE reactivity with allergen components especially Lol p 1 and 5 of RGP.

Participants will be provided with a copy of their spirometry results as it may be of interest to them and their doctors. Where permission is given the participant's nominated GP will be sent a copy of the results of testing.

13. Study Governance and Management

The overarching governance structure is outlined in the Figure below.
The Executive, made up of Principal Investigators, will establish committees for
(1) *Research Advisory* oversight which is an avenue for active consumer input on strategy and applicability;
(2) *Research Translation* for enabling the long-term translation of the research; and
(3) *Study Safety & Confirmation of Outcomes* which will monitor and validate outcomes (asthma exacerbations) and provide advice on study safety to the Executive.

The study will be operationally managed by a 0.8FTE post-doctoral researcher (Study Coordinator), including finalising the protocol, submission to ethics and governance, co-ordinating the customised app, building the database, and managing consumable and equipment purchases.
Clinical operations will be managed by 0.2FTE funded Nurse Co-ordinators who will lead the clinical implementation of the study at each hospital site.

14. APPENDIX

List of Attachments included:

Attachment	Document Name	Version Number	Date (e.g., Day-Month-Year)
1	CARISTA Baseline Data Collection Form	1.0	19 November 2024
2	Asthma Control Questionnaire (ACQ-5)	1.0	19 November 2024
3	Sino Nasal Outcome Test (SNOT-22)	1.0	19 November 2024
4	Total Nasal Symptom Score (TNSS)	1.0	19 November 2024
5	CARISTA Master Recruitment Flyer	1.0	19 November 2024
6	CARISTA Advertising text	1.0 2.0	19 November 2024 31 January 2025
7	CARISTA Website and Participant Expression of Interest text	1.0	19 November 2024
8	CARISTA Master Telephone Script	1.0 2.0	19 November 2024 31 January 2025
9	CARISTA Participant Contact and ID Form	1.0	19 November 2024
10	CARISTA Master Main PICF & Withdrawal of Consent Form	1.0 2.0	19 November 2024 31 January 2025
11	CARISTA Symptom Monitoring Platform Workflow	1.0 2.0	19 November 2024 31 January 2025
12	CARISTA Asthma Outcomes and Adverse Events Source Document	1.0	19 November 2024
13	CARISTA Investigator Agreement	1.0	19 November 2024

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16. AMENDMENT HISTORY

Amendment no.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made