UNIVERSITI PUTRA MALAYSIA

RHINOVIRUS-INDUCED ASTHMA EXACERBATIONS: IDENTIFICATION OF CYTOKINE/CHEMOKINE PROFILE VIA META-ANALYSIS

KOH SUE KIE

ABSTRACT

Rhinovirus-Induced Asthma Exacerbations: Identification of Cytokine and Chemokine Profile via Meta-Analysis

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Introduction: Rhinovirus (RV) infections are a major cause of common colds and asthma exacerbations. RV-induced asthma exacerbations are potentially caused by an increase in T helper 2 (Th2) cytokines and a defective production of T helper 1 (Th1) cytokines, especially interferons (IFNs). Primary bronchial epithelial cells (PBECs) and peripheral blood mononuclear cells (PBMCs) from asthmatics were reported to have deficient IFNs production, but the findings were inconsistent. **Objectives:** This study aims to use meta-analysis to compare the cytokine profiles between asthmatics and non-asthmatics before and after experimental RV infection (human experimental studies) and to compare RV-induced cytokine responses of PBECs and PBMCs derived from asthmatics and those of healthy controls (*ex vivo* studies). **Methodology:** Studies related to RV, asthma and cytokines were identified from three databases – PubMed, ScienceDirect and Scopus according to pre-defined selection criteria. A total of 19 eligible studies were then subjected to meta-analysis. **Results:** Meta-analysis comparing PBECs from asthmatics and healthy subjects revealed significant deficient production of antiviral cytokines IFN-β (-0.52, p= 0.008) and IFN-λ (-0.81, $p < 0.0001$) after *ex vivo* RV-infection. However, there was no significant difference in IL-6, IL-8, IP-10 and RANTES production. Meta-analysis comparing cytokine production of IFN-α and IP-10 production from PBMCs obtained from asthmatics and healthy controls did not reveal any significant difference when challenged with RV. In human experimental studies, post-infection IL-8 (1.08, $p < 0.001$) level was significantly higher while baseline IL-15 (-0.69, $p = 0.02$) was significantly lower in asthmatic group. However, there are only 2 studies included in the synthesis of data in each human experimental comparison group and thus more studies are needed to improve the power of the meta-analysis. **Conclusion:** Our meta-analysis revealed that significantly lower production of IFN-β and IFN-λ in PBECs may lead to increased risk of asthma exacerbations. Increased IL-8 after RV infection and lowered IL-15 production before RV infection in human subjects may also be tied to increased risk for asthma exacerbations. Deficient IFN production may result in reduced viral clearance while an imbalance of Th1 (e.g. IL-15) and Th2

(e.g. IL-8) cytokine may exaggerate chronic inflammation of the airways, subsequently leading to asthma exacerbations. Improving IFNs production and regulating pro-inflammatory cytokines may be a beneficial approach to prevent RV-induced asthma exacerbations.

Keywords: Rhinovirus, asthma, cytokine, IFN, meta-analysis

ABSTRAK

Eksaserbasi Asma Cetusan Rhinovirus: Pengenalpastian Profil Sitokin dan Kemokin melalui Analisis-Meta

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Pengenalan: Jangkitan Rhinovirus (RV) adalah penyebab utama selesema biasa dan eksaserbasi asma. Eksaserbasi asma cetusan RV mungkin disebabkan oleh peningkatan sitokin T helper 2 (Th2) dan kekurangan pengeluaran sitokin T helper 1 (Th1), terutamanya interferon (IFN). Sel-sel epitelium bronkus utama (*Primary bronchial epithelial cell*/PBEC) dan sel-sel mononuklear darah periferal (*Peripheral blood mononuclear cells*/PBMC) daripada pesakit asma dilaporkan mempunyai kekurangan pengeluaran IFN, tetapi penemuannya tidak konsisten. **Objektif:** Kajian ini bertujuan untuk menggunakan analisis-meta untuk membandingkan profil sitokin antara pesakit asma dan subjek kawalan yang sihat sebelum dan selepas jangkitan RV secara eksperimentasi (kajian eksperimen manusia) dan untuk membandingkan tindak balas sitokin yang dicetuskan oleh RV dalam kalangan PBEC dan PBMC yang berasal dari pesakit asma dan subjek kawalan yang sihat (kajian *ex vivo*). **Metodologi:** Kajian yang berkaitan dengan RV, asma dan sitokin dikenal pasti dari tiga pangkalan data - PubMed, ScienceDirect dan Scopus berdasarkan kriteria pemilihan yang telah ditentukan. Analisis-meta telah dijalankan kemudian ke atas 19 kajian yang berlayak. **Keputusan:** Analisis-meta yang membandingkan PBEC dari pesakit asma dan subjek kawalan sihat telah menunjukkan kekurangan pengeluaran sitokin antivirus IFN-β (-0.52, $p = 0.008$) dan IFN-λ (-0.81, $p < 0.0001$) selepas jangkitan RV secara *ex vivo*. Walau bagaimanapun, pengeluaran IL-6, IL-8, IP-10 dan RANTES tiada perbezaan yang signifikan. Analisis-meta yang membandingkan pengeluaran sitokin IFN-α dan pengeluaran IP-10 dari PBMC yang diperoleh dari pesakit asma dan subjek kawalan sihat tidak menunjukkan perbezaan yang signifikan ketika dicabar dengan RV. Dalam kajian eksperimen manusia, tahap IL-8 pasca-jangkitan (1.08, $p < 0.001$) jauh lebih tinggi manakala tahap IL-15 pada garis dasar (-0.69, $p = 0.02$) jauh lebih rendah dalam kalangan pesakit asma. Walau bagaimanapun, hanya ada 2 kajian yang termasuk dalam sintesis data dalam setiap kumpulan perbandingan kajian eksperimen manusia. Sehubungan dengan itu, lebih banyak kajian diperlukan untuk meningkatkan kekuatan and ketepatan

analisis-meta. **Kesimpulan:** Analisis-meta kami telah menunjukkan bahawa pengeluaran IFN-β dan IFN-λ yang lebih rendah dalam PBEC dapat meningkatkan risiko eksaserbasi asma. Peningkatan IL-8 selepas jangkitan RV dan kekurangan pengeluaran IL-15 sebelum jangkitan RV dalam subjek manusia juga mungkin berkaitan dengan peningkatan risiko eksaserbasi asma. Pengeluaran IFN yang kurang mungkin akan mengakibatkan pengurangan penyingkiran virus manakala ketidakseimbangan sitokin Th1 (IL-15) dan Th2 (IL-8) mungkin akan memburukkan keradangan kronik dalam saluran udara, seterusnya membawa kepada eksaserbasi asma. Peningkatan pengeluaran IFN dan mengimbangi pengeluaran sitokin pro-radang berpotensi menjadi pendekatan yang bermanfaat untuk mencegah eksaserbasi asma cetusan RV.

Kunci Utama: Rhinovirus, asma, sitokin, IFN, analisis-meta

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%	Percentage
Ab	Antibody
ADAM33	A Disintegrin and Metalloproteinase
APC	Antigen Presenting Cells
BAL	Bronchoalveolar Lavage
BEAS-2B	Primary and Immortalized Bronchial Epithelial Cells
CCL	Chemokine (C-C motif) Ligand
CD	Cluster of Differentiation
CI	Confidence Interval
CoV	Coronavirus
CXCL	Chemokine (C-X-C motif) Ligand
DC	Dendritic Cells
DZ	Dizygotic
ELISA	Enzyme-Linked Immunosorbent Assay
GWAS	Genome Wide Association Studies
HBEC	Human Bronchial Epithelial Cell
I^2	Heterogeneity
ICAM-1	Intercellular Adhesion Molecule 1
ICS	Inhaled Corticosteroids
IFN	Interferon
Ig	Immunoglobulin E

IL	Interleukin
IP-10	Interferon Gamma-Induced Protein 10
LDLPR	Low-Density Lipoprotein Receptors
MDA-5	Melanoma Differentiation-Activation Protein 5
MHC	Major Histocompatibility Complex
ml	millilitre
MOI	Multiplicity of Infection
mRNA	Messenger Ribonucleic Acide
MZ	Monozygotic
N/A	Not Available
NF-κβ	Nuclear Factor-κβ
ng	nanogram
NK	Natural Killer
NO	Nitric Oxide
OVA	Ovalbumin
PBEC	Primary Bronchial Epithelial Cells
PBMC	Peripheral Blood Mononuclear Cells
PFU	Plaque-Forming Unit
pg	picogram
PMID	PubMed Identification
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RANTES	Regulated on Activation, Normal T Expressed and Secreted
RevMan	Review Manager

RIG-I	Retinoic Inducible Gene I
RNA	Ribonucleic Acid
RSV	Respiratory Syncytial Virus
RV	Rhinovirus
SD	Standard Deviation
SEM	Standard Error of Mean
SMD	Standard Mean Difference
TCID	Tissue Culture Infective Dose
TGF-β	Transforming Growth Factor Beta
Th	T-helper
TLR	Toll-Like Receptor
UV	Ultraviolet
VEGF	Vascular Endothelial Growth Factor
α	Alpha
β	Beta
λ	Lambda

CHAPTER 1

INTRODUCTION

1.1 Background

Asthma is a major global burden, affecting more than 300 million individuals worldwide with increasing prevalence every year (Global strategy for asthma management and prevention, 1995; Bucchieri et al., 2002). Even though most asthma exacerbations are mild and self-limiting, report from Global Burden of Disease estimated around 420,000 deaths from asthma attack in 2016 alone (Strachan et al., 2018). Economically, asthma treatment costed United States of America US$64 billion in 2011, while annual treatment and medication cost ranged from US$150 to US$3000 per patient across the world (Sadatsafavi et al., 2018). Asthma is a major non-communicable, chronic inflammatory disease of the lower respiratory tract characterized by recurrent wheezing and breathing difficulty due to airway obstruction, bronchial hyperresponsiveness and airway remodelling (Renauld 2001). Rather than a single-sourced occurrence, asthma manifests as a combination of pathological damage and alteration to the airways exaggerated by imbalance in cytokine responses which mediates various cellular responses (Hansbro et al., 2008). The proposed mechanism of asthma exacerbation

is due to a dysregulated interplay of Th1 cytokine and Th2 cytokine production (Chung and Barnes, 1999).

Cytokines are extracellular signalling proteins that facilitate cell-cell interactions by relaying messages from one cell to another via downstream cascade of signals (Mahajan and Mehta, 2006). Upregulation and downregulation of certain cytokine could modify cellular responses towards different stimuli. Th2 cytokines are usually involved with humoral immunity and various repairing activities. An increase in Th2 cytokine in asthmatics could establish persistent inflammation and airway remodelling (Mahajan and Mehta, 2006; Desai and Brightling, 2009). Meanwhile, Th1 mediates cellular immunity and removal of pathogens. Decrease in Th1 production, specifically interferons (IFNs) in asthmatics impairs their microbicidal abilities, especially respiratory viruses which are major causes of exacerbations asthmatics (Hansbro et al., 2008; Hammond et al., 2015).

Respiratory viruses are a major cause of asthma exacerbations, detected in roughly 75% of individuals, with rhinovirus (RV) as one of the most common aero pathogens (roughly 60% of all respiratory viruses) to be detected (Message et al., 2008). RVs had been the major airway viral pathogen alongside coronaviruses (CoVs) and respiratory syncytial viruses (RSVs) to trigger a hyper-response of the airway epithelium (Tsukagoshi et al., 2013). Rhinoviruses (RVs) are a group of respiratory viruses capable of causing common colds in normal individuals, but they are also major potential trigger of asthma exacerbations (Papadopoulus et al.,

2004). An increase in Th2 cytokines with deficient Th1 cytokines production as the mechanism behind asthma exacerbations is widely agreed upon, but exactly how RV plays a role in causing an exacerbation in asthmatic patients is still debatable among researchers (Papadopoulus et al., 2004). Unlike other allergens that triggers excessive amount of proinflammatory cytokines which constricts the airways of an asthmatic patient, RV is capable of infecting the airway epithelium of both asthmatics and normal individuals (Dreschers et al., 2007). How asthmatics and healthy individuals produces cytokines in response towards this viral pathogen is the key of interest in this study.

Currently, there is no specific treatment to cure RV infections, where treatment options are limited due to abundance of RV serotypes and potential drug toxicities (Jacobs et al., 2013). Corticosteroid is currently the main therapy option to control asthma, but by any standards, asthma exacerbations are still recurring problems for every asthmatic even though they are on medications. This highlights the need to develop a targeted therapy that might be potentially helpful in modulating cytokine expression levels before RV-induced exacerbation occurs (Desai and Brightling, 2009). It is important to know which cytokine plays a key role in RV-induced asthma exacerbations in order to formulate a better treatment approach. For that, meta-analysis is used to compare all previous findings of cytokines profiles during RV-induced asthma exacerbations. Meta-analysis is a systematic approach to (1) analyse and summarize previous findings in a similar field, (2) assess the evidence of linkage between treatment and effect and (3) draw a critical estimation of overall effect (Haidich 2010). As such, meta-analysis is a suitable method of approach to identify the grey areas where the limitations and

disagreements of finding lies. Hence, this paper aims to find examine and compare cytokine profiles of asthmatics and non-asthmatics during RV-induced asthma exacerbation to hopefully uncover which cytokine can be a useful future marker of exacerbation and treatment.

1.2 Objectives

1.2.1 General Objective

This study generally aimed to investigate the cytokine profile of asthmatics and non-asthmatics during RV-induced asthma exacerbations.

1.2.2 Specific Objective

The specific objectives of this study is to use meta-analysis to compare cytokine profile (1) between asthmatics and non-asthmatics after experimental RV infection in human experimental studies and (2) between PBECs and PBMCs derived from asthmatics and those of healthy controls with and without RV infection in *ex vivo* studies.

1.3 Hypothesis

It is hypothesized that meta-analysis may reveal cytokine responses that contribute to RV-induced asthma exacerbations.

CHAPTER 2

LITERATURE REVIEW

2.1 Asthma

Asthma is a disease occurring as an interaction of both genetic and environmental factors. This chronic airway disease is indicated with the presence of a constellation of clinical features: (1) recurrent episodes of coughing, wheezing, chest tightness and breathing difficulty resulting from pathological airway obstruction; (2) broncho-hyperresponsiveness; and (3) airway inflammation (Renauld 2001; Corrigan 2008) following exposure to aeroallergens. The chronic inflammation present at the airways of an asthmatic patient results in a pathological alteration of the normal structure of the smooth muscle linings. This phenomenon is known as airway remodelling, where smooth muscle walls are thickened with a characteristic infiltration of eosinophils, mast cells and lymphocytes (Wills-Karp 1999).

Cellular level examination reveals a prominent player in the exacerbation of asthma: T-helper cells (Th), particularly $CD4^+$ Th2 cells. Th2 cells produces key cytokines such as IL-4, IL-5, IL- 9 and IL-13 crucial in mediating an IgE based inflammatory response by recruiting mast cells and eosinophils (Wills-Karp 1999;

Corrigan 2008). A heightened level of Th2 cells infiltration found at asthmatic airways following an antigen challenge indicated the role of Th2 in triggering an inflammatory response resulting in asthma (Robinson et al., 1992; Walker et al., 1992).

Genetic inheritance and interaction with environmental exposures are long proposed mechanism of the aetiology behind asthma (Holgate et al., 2007). Monozygotic (MZ) twin and dizygotic (DZ) twin studies are a convenient model of estimating the overall genetic contribution in the development of asthma by measuring the concordance rate (Holgate 1999). A study of 11,688 Danish twin pairs demonstrated 73% of asthmatic susceptibility are inherited, while the remaining are influenced by the environment (Skadhauge et al. 1999). MZ twins are also shown to have twice the elevated risk of asthma compared to DZ twins if one of the co-twins is affected, and males are more inclined to develop asthma (Thomsen et al., 2010). As hereditary is an important factor in asthma development, previous linkage analysis and genome wide association studies (GWAS) identified region 5q31–32 with genes encoding for IL-4, IL-5, IL-9, IL-13 and CD14 responsible for Th2 activities and atopic status (Wills-Karp et al., 2004; Bossé and Hudson, 2007; Vercelli 2008). The discovery of a disintegrin and metalloproteinase 33 (*ADAM33*) gene also strongly links asthma to genetic influence (Aierken et al., 2014).

2.2 Atopic Asthma

Asthma can be classified into phenotypes according to the time of onset, as well as its allergic status towards environmental factors (Saglani & Bush, 2008). Atopy is a status where an individual develops tendency to produce high levels of IgE, which are usually too exaggerated as a response towards common allergens, causing a humoral response that potentially leads to the development of diseases such as atopic dermatitis, rhino-conjunctivitis and eczema (Comberiati et al., 2017). The term "atopic asthma" specifically refers to a condition of airway inflammation hereditarily predisposed by high levels of IgE towards environmental allergens/antigens (Ali 2011). The allergic sensitization towards environmental allergens such as house dust mites, pollens, animal furs and certain respiratory viruses is a major risk factor in childhood asthma development (Frew 1999). The development of atopy is more prevalent among children, while adult populations have more significant cases of the "intrinsic" form of asthma. However, atopy does not necessarily translate into asthma, as studies showed that only 25-30% of sensitized children progressed into persistent inflammation in airway mucosa (Holt et al., 1999).

Atopic asthma is a two-step process, firstly by the build-up of immunological memory towards specified allergens, usually by naïve T-cells or Th0 cells which are exposed to the allergens by antigen presenting cells such as dendritic cells and macrophages (Kips 2001). This sensitization alone only increases the risk to develop respiratory hyperresponsiveness, but insufficient for

disease manifestation (Holt et al., 1999). To define atopic asthma, a secondary exposure to the allergen must be able to elicit a recurrent and persistent inflammation in the airway mucosa (Holt et al., 1999).

2.3 Asthma Exacerbation Pathophysiology

As mentioned above, asthma is a disease that requires two-stage activation of the immune system. The first step is when allergens enter the airway, which are ingested by antigen presenting cells (APCs), specifically dendritic cells (DCs) (Holgate, 2012). DCs will uptake the allergens, process them and present to naïve T cells (Th0) through antigen presenting on major histocompatibility complexes, MHC class I and II under the influence of IL-4 (Lambrecht & Hammad, 2012). This process promotes preferential differentiation of Th0 into Th2 cells, in a process known as sensitization (Ishmael 2011; Holgate 2012; Kudo et al., 2013).

Second stage involves activation of an asthma attack, or better termed as exacerbation. Exacerbation occurs as a failure of the immunomodulatory mechanism in the airway mucosa, resulting in the imbalance in cytokines, chemokines and antiviral protein production. It is noted that there is an increase in pro-inflammatory cytokines such as IL-4, IL-5, IL-13 mainly by Th2 cells and more recently, IL-17A IL-17F, IL-22 by Th17 subtype cells for neutrophil recruitment and infiltration (Kudo et al., 2013). Immune cells-recruiting

chemokines such as CXCL8 (IL-8), CCL11 (eotaxin), CCL5 (RANTES) are released by epithelial cells, eosinophils, T-cells and mast cells (Barnes 2008). These chemokines are attractants of more inflammatory cells such as leukocytes, causing a surge of immunomodulatory cells at the site of inflammation (Barnes, 2008). As a result, accumulation and infiltration of immune cells exacerbates the inflammation in the airway mucosa. In cases of respiratory virus infection, interferons are also notably reduced in atopic asthmatics compared to healthy individuals (Wark et al., 2005). Th1 cells produces useful cytokine to fight against infection. NO is useful in inhibiting activation of Th1 and Th2, but the effect is more pronounced in the former (Bingisser et al., 1998; Holt et al., 1999). This in turns skew the cytokine production towards Th2 types (Liew, 1995; Corne & Holgate, 1997).

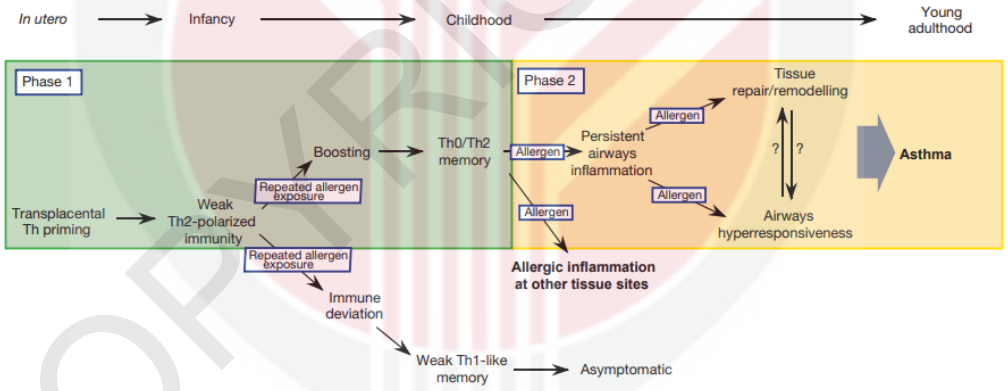

Figure 2.1. The two stages of allergic asthma, from initial sensitization to the development of chronic asthma (Holt et al., 1999)

IgE is the key player in eliciting an over-reactive immune response in asthma. Circulating IgE levels are higher in asthmatic patients, and they are the main reason to broncho-hyperresponsiveness in asthma (Postma 1995). IL-4 and

IL-13 produced by Th2 cells promote the switching of IgM production to IgE production when B-cells are stimulated (Corrigan 2008). The increased amount of IgE will bind to mast cells via FcεRI, a high affinity receptor for IgE. The cross-linkage triggers the release of a cocktail of proinflammatory proteins, such as histamine, prostaglandin, leukotrienes, and cytokines such as IL-4, IL-13 and TGF-β (Renauld et al., 2001). The immediate effects of the proinflammatory proteins are vasoactive in nature, inducing smooth muscle constriction and increasing mucosal production, which prepares the airway for an inflammatory infiltrate (Renauld et al., 2001).

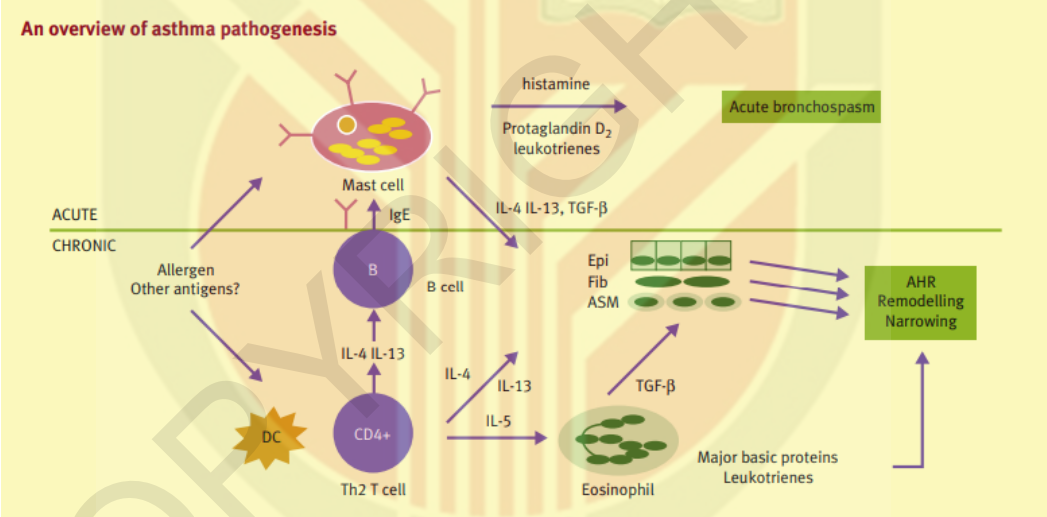

Figure 2.2. The production of cytokines and the pathogenesis of asthma (Corrigan 2008).

The exaggeration of the immunomodulatory response stimulated by an allergen results in a downstream signalling pathway of cytokines. The T-cell mediated response is unbalanced. Prolonged inflammation creates a continuous cycle of inflammation and attempt for wound healing (Holt et al., 1999). The

epithelium also releases growth factors such as vascular endothelial growth factor (VEGF) to promote angiogenesis and oedema, transforming growth factor β (TGF-β) for fibroblast proliferation and reforming the extracellular matrix (Barnes, 2008). These changes the cellular framework and environment, propagating airway remodelling.

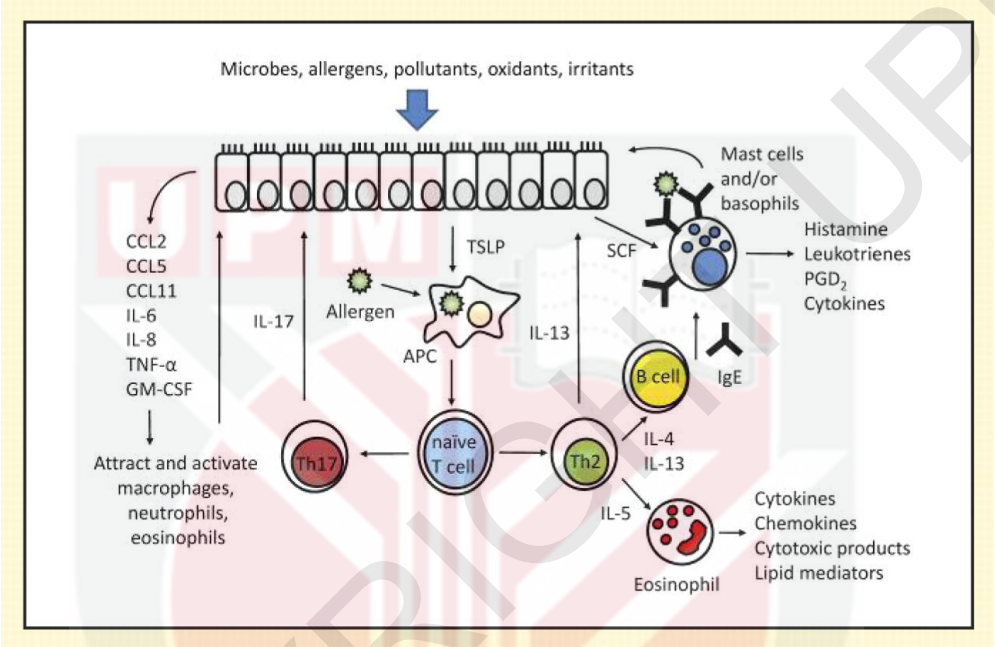

Figure 2.3. The interaction of immunoregulatory cells through cytokine/chemokine interaction (Ishmael 2011).

2.4 Rhinovirus

Rhinovirus is a member of the family *Picornaviridae*. Its basic structure consists of a single stranded, positive-sense RNA, encased in an icosahedral-shaped capsule (Jacobs et al., 2013). RV mainly infects the lower respiratory

airway with a lowered optimum temperature at 33℃ (Jacobs et al., 2013; Corne and Holgate, 1997). Other than infecting host lower respiratory airways, RV also demonstrated the ability to infect primary bronchial epithelial cells under *ex vivo* settings very well after experimental inoculation (Kelly and Busse, 2008). The major group, almost 90% of RVs infect the lung epithelium mainly through the intercellular adhesion molecules (ICAM-1) located on bronchial epithelium surface (Papadopoulos et al., 2004). ICAM-1 is proposed to be upregulated in asthmatic patients, which might be an underlying factor for exaggerated inflammatory response towards RV infection (Wegner et al., 1990). Studies showed that PCR-based examination revealed a significant proportion (50-60%) of viral induced asthma exacerbation is due to RV infection (Papadopoulos et al., 2004; Hershenson, 2013).

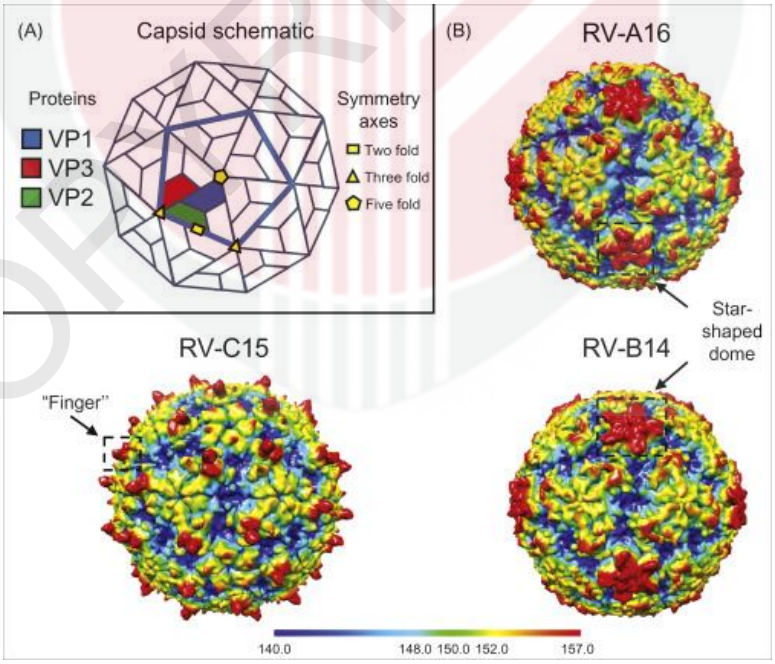

Figure 2.4: Schematic illustration of capsid and 3D model of RV (Esneau et al., 2019).

2.5 Mechanism of RV-Induced Asthma Exacerbation

In an episode of RV-induced asthma exacerbation, viral load was expected to be greater in asthmatic patients than healthy controls. However, study by Kennedy et al. (2014) measured only similar amount of viral concentration between these two groups. The cytopathic effect of RV is more negligible compared to other respiratory viruses such as influenza and coronaviruses (Winther et al., 1984; Frankael et al., 1995; Papadopoulos et al., 2004). However, another study argued that the damage to the airway epithelium in an asthmatic patient might be more vulnerable towards RV infection (Campbell et al., 1993).

This infers that differential cytokine responses towards RV infection is the cause for RV-induced asthma exacerbation. (Hammond et al., 2015). Replication of RV produces RNA particles which can be detected by the innate immune receptors such as retinoic acid inducible gene I (RIG -I), melanoma differentiation-activation protein 5 (MDA-5) and toll-like receptors (TLRs) (Yoneyama et al., 2004; Ritchie et al., 2015). After binding with the receptors, transcription factors such as nuclear factor-kB (NF-kB) are then produced to direct the nucleus of the infected bronchial epithelium to produce cytokines and chemokines in response of the spreading RV (Laza-Stanca et al., 2006; Ritchie et al., 2015). Such chemokines include Th1 cytokines, Th2 cytokines and interferons (Ritchie et al., 2015).

Various studies demonstrated RV infection will lead to an increase in cytokines and chemokines expression, notably IL-8, IL-6, RANTES (Subauste et

al., 1995; Gern et al., 2003). Chemo-attractants of eosinophilic origin, eotaxin and eotaxin-2 were induced in *in vivo* studies using BEAS-2B cell lines when RV is introduced (Papadopoulus et al., 2004, Papadopoulus and Johnston, 2001).

Interferon (IFN) is identified as one of the key factors for an effective viral clearance. The protective effects of interferon come from its ability to interfere with the viral replication processes and producing a heightened counter-viral response by recruiting immune cells to perform various viral clearance tasks (Fensterl and Sen, 2009; Ritchie et al., 2015). RV can to elicit an asthma exacerbation in atopic individuals with a heightened pro-inflammatory cytokine production and a lowered interferon production (Berger, 2000). This is supported by various studies, where interferons of Type 1 (IFN-α/β), Type II (IFN-γ) and Type 3 (IFN-λ) are reportedly deficient in production by bronchial epithelium and peripheral mononuclear cells (Gavala et al., 2014). In normal and healthy circumstances, Type III IFNs was demonstrated to be the principal IFN of innate antiviral response of BECs. Type I IFN-α was released by PBMCs while Type I IFN-β can be released by both cell types as an antiviral response in vitro (Khaitov et al., 2009). The combined effect of proinflammatory cytokines, massive amount of immunomodulating cell infiltration and reduced viral clearance, increase in vascular leakage and mucous secretion, together with an asthmatic's pre-existing inflamed and narrow airway leads to exacerbation (Steinke and Borish, 2016).

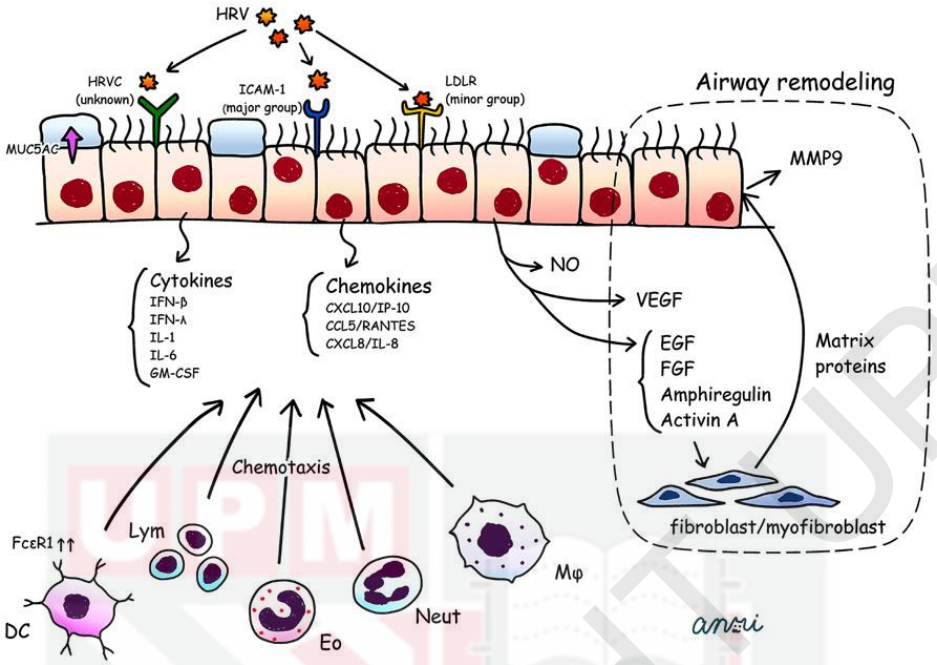

Figure 2.5: Rhinovirus-induced asthma exacerbation model (Saraya et al., 2014)

2.6 Inflammatory Cytokines and Interferons in RV-Induced Asthma Exacerbations

Originating from T-lymphocytes, cytokines can be sub-divided into 2 groups, Th1 cytokines and Th2 cytokines (Berger, 2000). Th1 cytokines enables cellular immunity, which are mainly involved in the destruction of intracellular parasites, elimination of cellular antigens, modulation and sustenance of autoimmune responses. The prime examples of Th1 cytokine are IFN-γ and IL-12. Th2 cytokines has anti-inflammatory effects and plays a key role in humoral immunity through the production of antibodies, which explains its role in IgE-

mediated eosinophilic response in atopic asthma. Examples of Th2 cytokines are IL-4, IL-5, IL-13. There is a mutual inhibitory effect observed in the selection of Th1 and Th2 cell type differentiation (Berger, 2000). Th1 cells produces IFN-γ that is antagonistic in the differentiation pathway of naïve T cells into Th2 cell types. In return, propagation of Th1 cell populations is inhibited by IL-4 produced by Th2 cells (Hershenson, 2013).

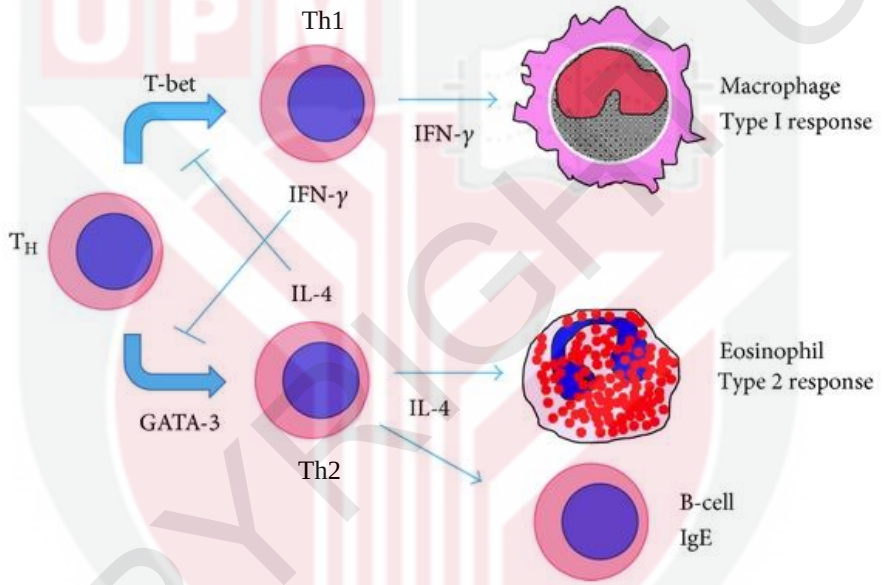

Figure 2.6. The mutual inhibitory effects of Th1 and Th2 propagation selection (Hershenson, 2013).

Conflicting cytokine profile findings were reported in various studies on RV-induced asthma exacerbations. The general accepted mechanism of exacerbation is due to deficient interferons levels and a heightened pro-inflammatory cytokine response. This is supported by a few studies that reported

deficient IFN-β and IFN-λ production in human bronchial epithelial cells (HBECs) and bronchoalveolar lavage (BAL) cells after inoculation with RV (Wark et al., 2005; Contoli et al., 2006; Wark et al., 2009). Viral load was also found to be increased together with a decreased IFN production (Wark et al., 2005; Contoli et al., 2006; Wark et al., 2009). Despite this, there are a few studies whose findings rebutted this mechanism. IFNs levels were the same, or even higher in concentration among asthmatics (Gavala et al., 2013). Type III IFN-λ level is higher is asthmatic children when challenged with RV, along with worse exacerbation symptoms (Miller et al., 2012). Study by Sykes et al. (2013) too, was unable to prove that type I or type III IFNs were deficient in HBECs under similar experimental settings as previous studies.

Proinflammatory cytokines such as IL-4, IL-5 and IL-13 upregulate cell interaction molecules located on epithelial cells. This act promotes chemotaxis to encourage eosinophil binding and release of inflammatory proteins (Kelly and Busse, 2008) and are the focal point of atopy asthma exacerbations. Although the role of Th2 cytokines in initiating an atopic exacerbation is widely agreed upon, studies found that IL-4 induction can only be found in asthmatic subjects, while IL-13 is induced in both healthy and atopic asthmatics during an exacerbation (Papadopoulos et al., 2004).

2.7 Meta-Analysis

Meta-analysis is a systemic review and analysis of previous studies (usually the effect of treatment and risk factor), compiling and comparing the data of previous findings of similar interests, then generating a new set of data that estimates the overall effects of previous findings (Haidich, 2010). An important concept in meta-analysis is the need for critical and logical appraisal of search results according to the field of interest (Gopalakrishnan and Ganeshkumar, 2013). The results generated are quantifying and gives a more precise estimation of cause and effects, especially in experimental and clinical settings (Mikolajewicz and Komarova, 2019). This allows researcher to have a greater overview on the effects of different treatments on various responding groups.

The scientific proving any medicine, facts and theories require meticulous planning and detailed conduct, together with an experimental design or study model that can give concluding and convincing results. Thus, evidence-based medicine is highly prized (Guyatt et al., 1995; Sacket et a., 1996). Conventional research and experiments are conducted at various times across the world, with different set ups. The subtle differences of each independent research may give rise to contradicting results. As such, meta-analysis sits on top of the hierarchy of evidence, because it is an integrative method that combines the results of previous independent studies (Haidich, 2010).

Figure 2.7: The hierarchy of evidence (Haidich, 2010).

Meta-analysis can be useful in addressing the conflicting situation of difference in cytokine profile during RV-induced asthma exacerbations between healthy controls and atopic asthmatics. This is because meta-analysis employs the element of statistical analysis in testing the hypothesis of a study (Mikolajewicz and Komarova, 2019). The strength of meta-analysis comes from critical protocols in the setting of the study. Initially, a thorough literature search according to the PRISMA guideline sets up the framework. Potential studies are screened according to selected criteria only (Liberati et al., 2009). Results of each contributing literature are weighted accordingly to the size of their study (Cohn and Becker, 2003). Heterogeneity is also measured across the analysis to determine a suitable statistical model for the estimation of effects (Cohn and Becker, 2003). The presence of bias from each literature is quantified to represent the reliability of the analysis (Liberati et al., 2009).

Heterogeneity or I^2, is the degree of variability between study results, an indicator of inconsistency found between studies (Ryan 2016). The greater the percentage of I^2, the greater the degree of heterogeneity. The idea is based on how much of the confidence level (CI) of each study overlap with each other. The greater the degree of overlapping, the lower the heterogeneity (Higgins and Green, 2011). Researchers can actually choose to abandon the meta-analysis if there is a presence of considerable heterogeneity. However, adjusting the effect model according to the heterogeneity is a method to cope with. This is because each study has different variability in design and complexity, therefore optimizing the effect model rather than outright rejecting the meta-analysis due to prominent heterogeneity is preferred (Sedgewick 2015; Ryan 2016).

2.8 Publication Bias

Publication bias is a situation where positive or favourable outcomes receives more publications than the negative and unfavourable outcomes. It creates a false sense of optimism, that may lead to critically incorrect conclusion of the situation (Sutton et al., 2000; Rothstein et al., 2006). Publication bias is addressed in a two-step method: (1) to detect the presence of the bias and (2) to determine the implications of publication bias (Rothstein et al., 2006; Fragkos et al., 2014). The first step employs visual assessment such as funnel plot and statistical tests to estimate the presence of bias, followed by secondary approaches such as Rosenthal's fail-safe N and the trim and fill method to determine the extent of

corrective measurements needed to correct the publication bias (Sutton et al., 2000; Fragkos et al., 2014; Kow et al., 2019).

Funnel plot is a graphical display of association between treatment effect and sample size to detect publication bias (Bradburn, n.d.). This is based on the idea where smaller studies will need greater treatment effect in order to be established as significant. Therefore, the scattering of the studies across the plot lies with the fact that larger studies will have better estimation of precision, while smaller studies are more prone to minor errors which affect the treatment effect, and therefore are plotted lower and more spread out across the funnel plot (Sterne et al., 2000). If the studies are plotted asymmetrically, it indicates the absence of negative studies and presence of publication bias (Sterne et al., 2000).

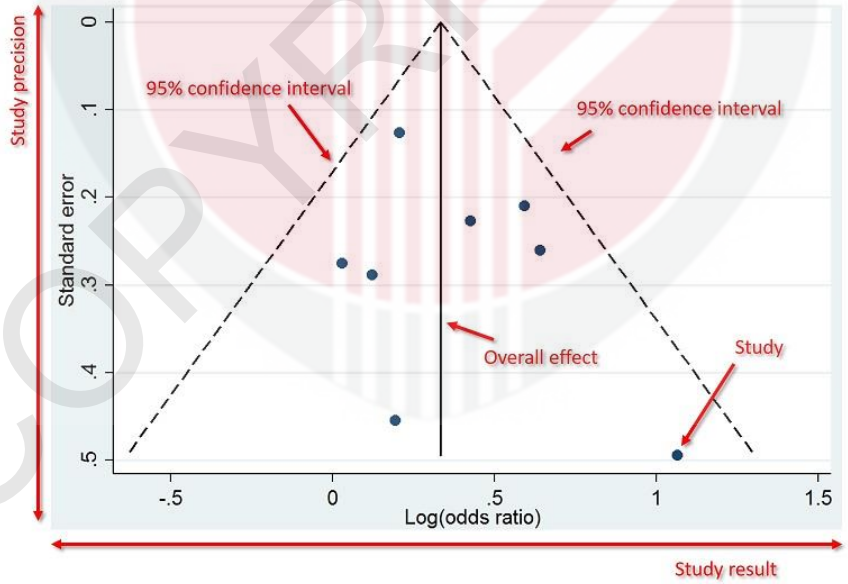

Figure 2.8. Basic components of a funnel plot and how to read them (Bradburn, n.d.). Larger studies have better precision, so they will congregate on the tip of the funnel plot, while smaller studies will scatter nearer towards the bottom.

Funnel plot alone is insufficient to conclude the presence of publication bias. Heterogeneity of treatment effects and complexity of trials could lead to various deviation regardless of the actual presence of bias (Sterne et al., 2000). Therefore, quantifying methods to detect publication bias were proposed: Egger's regression test and Begg's test (Duval and Tweedie, 2000). Both test questions the relativity of study estimation to study size. Begg's test ranks the correlation of variance and effect size. If a significant correlation is found between effect size and their sampling variance, publication bias is hinted (Begg and Mazumdar, 1994; Bland 2006). Egger's test uses a regressing approach, where the regressed standard effect is expected to be zero when there is an absence of publication bias (Egger et al., 1997; Lin and Chu, 2018). While useful in quantifying publication bias, these two tests have weak significance when the number of studies is few. Due to this limitation, it is often that only p-value of these tests were reported, but not the degree of correlation or magnitude of regression (Lin and Chu, 2018).

Rosenthal's fail-safe N is an approach to address possible sampling error in the process of research, where it is frequently called the "file-drawer effect" (Rosenthal 1979, Orwin 1983). It is based on the Stouffer Method to combine p-value through the computation of Z-score according to Monsteller and Bush (1954). This test estimates how many zero-effect or null effect studies can be added to the given overall outcome before the significance of the meta-analysis is reduced to α level (e.g.: $p = 0.05$) (Rosenthal 1979). Small fail-safe N number may indicate the susceptibility towards publication bias.

Trim and fill method is an interesting approach to correct the overall effect size of funnel plot while testing for the presence of publication bias (Duval and Tweedie, 2000). Trim and fill method takes in assumption that studies on the extreme left and right side of an overall effect (funnel plot) will be suppressed. First step to approach the overall effect is to "trim" away studies that affect the symmetry of a funnel plot. By assuming the remaining studies are unlikely to be affected by publication bias, the method then "fills" the funnel plot with missing studies (either positive or negative studies) to correct the funnel plot of skewness (Shi and Lin, 2019).

CHAPTER 3

METHODOLOGY

Meta-analysis

3.1 Protocol and Registration

The methods for conducting the meta-analysis were performed according to the PRISMA statement (Liberati et. al, 2009). Due to the nature of the studies included, only publication bias assessment was performed to evaluate the risk of bias across studies and no assessment of the risk of bias in individual studies using Cohrane's risk of bias tool was conducted (Kow et al., 2019).

3.2 Eligibility Criteria

3.2.1 Types of Studies

Ex vivo studies where primary bronchial epithelial cells (PBECs) or peripheral blood mononuclear cells (PBMCs) of human origin were infected with rhinoviruses *ex vivo*; and human experimental studies where subjects were experimentally infected with rhinoviruses.

3.2.2 Types of Participants

Each study must have 2 groups of participants; asthmatic subjects and non-asthmatic healthy subjects. No age, gender or racial restrictions were imposed. Asthmatic subjects were classified into atopic asthmatics and non-atopic asthmatics. Asthma is a respiratory disorder, characterised by inflammation and obstruction of the airflow, leading to symptoms such as recurrent wheezing and shortness of breath (Pillai et al., 2011). Atopic asthma and non-atopic asthma are classified based on the presence or absence of allergic condition with episodes of asthmatic symptoms with precipitation from specific sensitizations to various aeroallergens that leads to characteristic immunoglobulin E (IgE) antibodies associated eosinophilic airway inflammation respectively (Comberiati et al., 2017). The atopic status can be determined through serology or skin prick tests with a careful assessment of the patient's history (Ali, 2011).

3.2.3 Types of Intervention

Experimental studies comparing the effects of rhinovirus infections on cytokine responses between asthmatics and non-asthmatic healthy controls were included in the meta-analysis. All RV serotypes were included. There was no restriction on RV dosage used for infection.

3.2.4 Types of outcome measures

The outcome measures were the level of any cytokine upon RV infection for *ex vivo* studies and the pre- and post-infection levels of any cytokine for human experimental studies. The parameters must be in measurable concentration of protein (pg/ml, ng/ml). Outcomes in the form of fold change and gene expressions (mRNA) will be excluded.

3.3 Search Strategy and Data Source

Related articles were obtained from 3 electronic search engines: PubMed, Scopus and ScienceDirect. The keyword for the search was "rhinovirus* AND asthma* AND (cytokine* OR chemokine* OR interferon* OR antiviral*)". Language and publication dates were not restricted. Filters were applied to select articles, editorials and letters only. The last search on the databases was performed on 26th November 2019. The studies were screened according to the eligibility criteria by the first reviewers (Sue Kie Koh, Kong Yen Liew, Matthew Kah Lup Ng, Suet Li Hooi). Eligible studies were confirmed by the second reviewer (Kong Yen Liew).

3.4 Study Selection

Results from three databases were sorted out according to their publication dates. Duplicates were removed if they have the same author, title, and PubMed identification (PMID). For records without PMID, manual screening for identical author and title was performed to remove the duplicates. The remaining records were screened according to the eligibility criteria. The eligibility assessment was accomplished via independent reviews of the abstracts in an unblinded but standardized manner by 4 reviewers. Disagreements between reviewers were resolved by reasoning and consensus. After screening based on the title and abstract was done, eligible studies are subjected to full-text screening to further determine their relevancy. Studies without meeting all the eligibility criteria were removed. The flow of study selection was done by referring to the PRISMA flow diagram. Reasons for excluding the studies will be recorded.

3.5 Data Extraction

The levels of cytokines were obtained from data tables, bar charts, linear graphs, or dot plots. Data from the graphs were extracted using Image J software version 150. Mean and standard deviations (SD) were obtained from bar charts or linear graphs by measuring the lengths against the y-axis value. For studies reporting the findings in the form of median and interquartile range, the values were converted to mean and standard deviation as described previously (Wan et al., 2014). Values from dot plots were manually averaged after measuring the value of each dot with Image J. For studies reporting standard error of mean (SEM) instead of SD, the values were converted into SD using Review Manager (RevMan) version 5.3 for Cochrane Systematic Review.

Other than the levels of cytokines, the characteristics of each study were documented and classified into three categories, which are *ex vivo* PBEC, *ex vivo* PBMC and human experimental studies. The following characteristics will be obtained from each included study: subject demographics (gender, age), allergic status, rhinovirus (subtype and dose for experimental infection), infection duration, type of biological samples, detection parameters and assay, and additional remarks (if present).

3.6 Data Analysis and Result Synthesis

First, the studies were classified into three categories: (1) *ex vivo* PBEC, (2) *ex vivo* PBMC and (3) human experimental studies. Meta-analysis was conducted on each cytokine only if 2 or more studies reported the same cytokine within the same category. For each cytokine, mean, standard deviation (pg/ml) and the number of subjects of both groups (asthmatics vs healthy control) were inserted into the software RevMan 5.3. Standardized mean difference (SMD) of cytokine level of two groups for each study are compared within 95% confidence interval. The overall effects were determined with either fixed-effect or random-effects statistical model based on the heterogeneity (I^2) between the studies. The random-effects model was used when heterogeneity was significant ($I^2 > 75\%$), while fixed-effect model was used when heterogeneity was not significant ($I^2 < 75\%$). After adjusting the statistical model, the significance p-value was recorded. If $P > 0.05$, the results were deemed insignificant. If $p < 0.05$, the results were deemed significant.

3.7 Publication bias assessment

Publication bias of each cytokine in the meta-analysis was conducted with Meta Essentials Microsoft Office Workbook. The presence of publication bias is determined by Funnel plot, Begg's test, Egger's Regression test, Rosenthal's Fail-safe N and Trim and Fill method (Kow et al., 2019).

CHAPTER 4

RESULT

Meta-Analysis

4.1 Literature Search

The keywords used as search item were “rhinovirus* AND asthma* AND (cytokine* OR chemokine* OR interferon* OR antiviral*)”. A total of 2158 studies were retrieved from 3 databases whereby 505 were from PubMed, 511 were from Scopus and 1142 were from ScienceDirect. 494 studies were removed as duplicates through PMID serial number comparison and manual screening of similar titles and abstracts. The remaining 1664 studies were screened by title and abstract according to the eligibility criteria. After screening, 1632 studies were excluded, and the remaining 32 studies were further reviewed in full-text assessment. Thirteen studies were excluded as they did not meet the described eligibility criteria. Nineteen remaining studies were subjected to meta-analysis in their respective category of study.

Figure 4.1. Flow of literature search according to the PRISMA guidelines

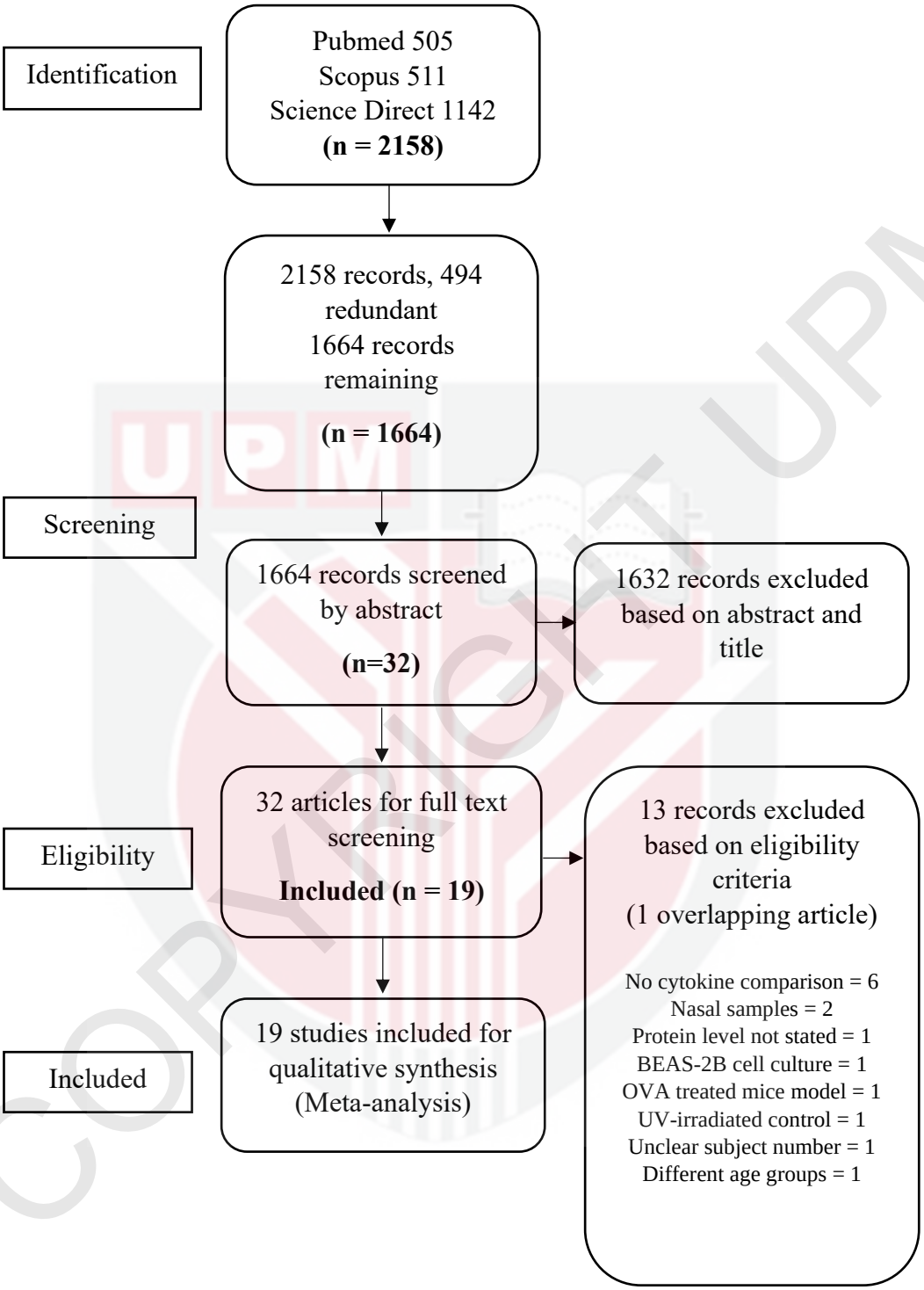

Table 4.1 depicts the characteristics of the 19 studies included in meta-analysis. The studies were classified according to the type of study and their publication year. Among them, 12 papers (63.16%) were *ex vivo* PBEC studies, 3 papers (15.79%) were *ex vivo* PBMC studies, while 4 papers (21.05%) used human experimental study model. Only 1 study (5.26%) was published before 2000, 5 studies (26.32%) in between 2000-2009 and 13 studies (68.42%) included were published in between 2010-2019. The trend may be attributed to the increasing demand to elucidate the cytokine responses during a RV-induced asthma exacerbation. Almost 70% of the studies were reported to be between the year 2010-2019, which indicate cytokine production as a promising revelation of the complex mechanism of RV induced asthma exacerbations.

Table 4.1. Characteristics of included studies categorized based on the type of study and year of publication.

Category	Number of studies	Percentage (%)
Type of Study		
Ex vivo PBEC	12	63.16
Ex vivo PBMC	3	15.79
Human experimental	4	21.05
Year of Publication		
1990-1999	1	5.26
2000-2009	5	26.32
2010-2019	13	68.42

4.2 Data Extraction and Organization

Detailed characteristics of the included studies were illustrated for *ex vivo* PBEC, *ex vivo* PBMC and human experimental studies respectively. The subject demographics, cytokines of interests, type of studies and the reference were included.

Table 4.2.1 Summary of included *ex vivo* PBEC studies examining RV-induced cytokine responses

Author, year	Subjects allergy status	N (male – female)		Age (mean)	RV		Infection duration (hours)	Cytokines measured	Assay	Remarks
		M	**F**		**type**	**dose**				
Wark et al., 2005	Moderate atopic asthmatic (ICS treated) Mild atopic asthmatic (ICS naïve) Non-atopic healthy controls	10 6 6	4 4 4	32 (21-58) 32 (12.6) 29 (24-38)	RV16 RV1B	2 MOI	48 hours (for all 3 sub-categories	IFN-β	ELISA	3 data sets were obtained: (1) ICS treated + RV16 (2) ICS treated + RV1B (3) ICS naïve + RV16
Contoli et al., 2006	Asthmatics Non asthmatic healthy controls	5 4	6 3	31.2 (21-50) 27.8 (28-31)	RV16	2 MOI	48	IFN-λ	ELISA	

Wark et al., 2007	Corticosteroid naive asthmatics Non asthmatic healthy controls	4 4	6 6	30 (16-74) 31 (range not stated)	RV16	not stated	48 (peak)	IL-6 IL-8 IP10 RANTES	ELISA	All subjects were non-smokers
Lopez-Souza et al., 2009	Atopic asthmatics Non-atopic healthy controls	4 3	2 2	33 (24-50) 37 (32-46)	RV16	1×10^{6} TCID50 / 1 MOI	48	IL-6 IP10 RANTES	LINCOplex	
Wark et al., 2009	Asthmatics Non asthmatic healthy controls	2 2	2 2	36.5 (28-44) 38.75 (28-50)	RV1B	1 MOI	48	IFN-β	ELISA	Age and gender of subject 2 and 4 of healthy control not specified. All atopic asthmatics have daily dose of ICS
Bochkov et al., 2010	Asthmatics Non asthmatic healthy controls	4 3	5 6	25.4 (19-35) 252 (19-41)	RV1A	10PFU/cell	16	IL-6 IL-8	Sandwich ELISA	
Baraldo et al., 2012	Atopic asthmatics Non-atopic healthy controls	4 3	2 2	5 (2-13) 5 (2-7)	RV16	1 MOI	48	IFN-β	ELISA	Children IFN-λ and IL-8 protein level not measured. Only IFN-β protein level measured

Edwards et al., 2013	Severe therapy resistant atopic asthmatics Non atopic healthy controls	6 7	5 4	11 (9-15) 7 (2-15)	RV16 RV1B	2 MOI (Wark et al., 2005)	24	IFN-β IFN-λ IL-8	ELISA	Two RV serotypes used
Sykes et al., 2014	Atopic asthmatics Non atopic healthy controls	8 5	2 7	34.2 (±8.4) 28.25 (±11.1)	RV1B	1 MOI	48	IFN-β IFN-λ	ELISA	
Parsons et al., 2014	Asthmatics Non asthmatic healthy controls	3 4	9 4	54.5 (SD 9.9) 55.9 (SD 12.3)	RV1B	20 MOI	24	IFN-λ IL-6 IL-8 IP10	ELISA	
Bai et al., 2016	Asthmatics Non asthmatic healthy controls	0 4	6 2	46.3 (9-64) 41.7 (13-62)	RVA16	0.69x TCID50/mL	24	IL-6 IL-8 IP10 RANTES	Luminex 42 plex	Lungs from deceased donors
Mowska et al., 2018	Atopic asthmatics Non atopic Asthmatics Non atopic healthy controls	5 2 5	1 2 4	29 (±6.1) 40.25 (±10.69) 31.78 (±14.62)	RV1B	0.1 MOI	48	IFN-λ IP10	ELISA	Asthmatic patients are divided into atopic and non-atopic asthmatics

Table 4.2.2. Summary of included *ex vivo* PBMC studies examining RV-induced cytokine responses.

Author, year	Subjects allergy status	N (male – female)		Age (mean)	RV		Infection duration (hours)	Cytokines measured	Assay	Remarks
		M	**F**		**type**	**dose**				
Davies et al., 2011	Atopic asthmatics Non-atopic healthy controls	0 1	12 11	30.00 (28.33-40.91) 43.46 (33.54-50.81)	RV16	1 MOI	24	IFN-α IP10	ELISA	Six asthmatic subjects used corticosteroid One asthmatic subject used ICS spray Healthy group slightly older but all are pre-menopausal
Sykes et al., 2012	Atopic asthmatics Non-atopic healthy controls	16 9	6 11	33 (±1.713) 38.4 (±2.426)	RV16	1 MOI	24	IFN-α	ELISA	
Pritchard et al., 2014	Atopic asthmatics Non-atopic healthy controls	11 10	11 10	33.83 (±12.9) 35.3 (±12.6)	RV16	5 MOI	24	IFN-α IP10	ELISA	Half of the asthmatic subjects were prescribed ICS All withheld corticosteroid 24h prior to blood sampling

Table 4.2.3. Summary of included human experimental studies examining RV-induced cytokine responses.

Author, year	Subjects allergy status	N (male – female)		Age	RV		Time of sample taken	Sample type	Cytokines measured	Assay	Remarks
		M	**F**		**type**	**dose**					
Fleming et al., 1998	Atopic asthmatics Non atopic healthy controls	5 2	6 8	34.7 (24-56) 29.3 (19-43)	RV16	1000 TCID50/mL	Baseline and acute cold	Sputum	IL-8	EIA	Asthmatics required to be only on beta-agonist inhalation treatment
Laza-Stanca et al., 2011	Asthmatics Healthy controls	9 14 (M/F ratio unknown)		unclear	RV16	5 MOI	Baseline and 48 hours	Bronchial alveolar lavage fluid	IL-15	ELISA	Subject allergic status not given
Rohde et al., 2014	Mild atopic asthmatics Non atopic healthy controls	2 8	8 7	22.0 (SD 2.8) 26.9 (SD 8.9)	RV16	10000 TCID50	Baseline and Day 4	Bronchial alveolar lavage	IL-8	Luminex analysis	
Hansel et al., 2014	Asthmatics Non-asthmatic healthy controls	13 7	15 4	36 (±11) 31 (±12)	RV16	100 TCID50	Baseline and Day 4	Bronchial mucosal lining fluid	IL-15	Multiplex immunoassay	All non-smokers, without Ab towards RV-16. Asthmatics (n=25); Healthy at baseline (n=11), Healthy at Day 4 (n=10)

4.3 Schematic Diagram of Meta-Analysis

4.3.1 *Ex vivo* PBEC Studies

Figure 4.3.1 – 4.3.6 showed the forest plots constructed from the meta-analysis of cytokines from *ex vivo* PBEC studies. Analysis of the standard mean difference across the studies were used for the large variation of mean findings. For each cytokine, the heterogeneity across different studies determined the effect model to be used. A total of 6 cytokines were analysed under this category, namely IFN-β, IFN-λ, IL-6, IL-8, IP-10, and RANTES. Among the 6 cytokines, only IFN-β and IFN-λ production by PBECs of asthmatic subjects were found to be significantly impaired compared to healthy individuals in the *ex vivo* study model.

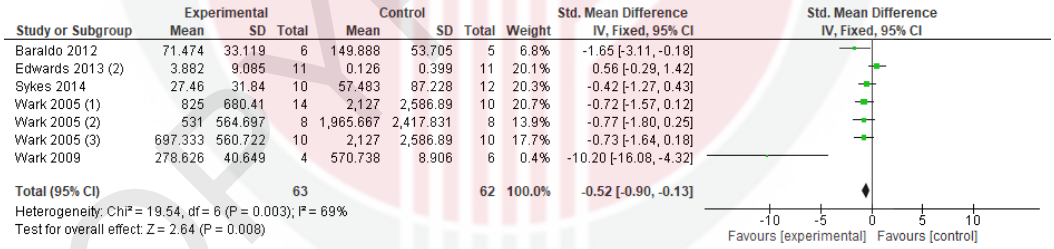

Figure 4.3.1. Forest plot of the meta-analysis of IFN-β levels from 7 studies using PBEC model infected with RV *ex vivo*. I^2 = 69% indicated insignificant heterogeneity, and thus fixed-effects model was used. There was significant difference in the production of IFN-β by PBECs between the experimental group (asthmatics) and the control group (healthy controls) after *ex vivo* RV infection, with $p = 0.008$ ($p < 0.05$) at 95% CI.

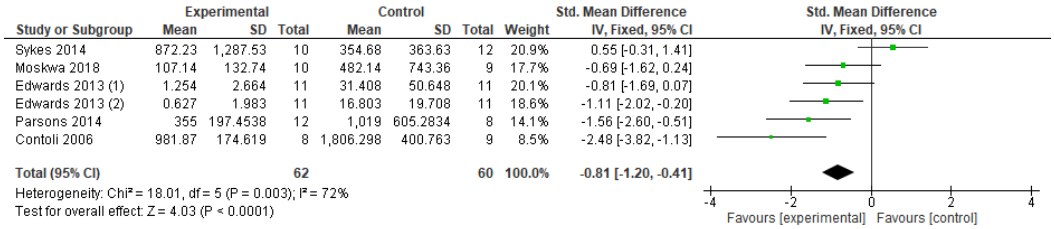

Figure 4.3.2. Forest plot of the meta-analysis of IFN-λ from 6 studies with RV infection on *ex vivo* PBEC model. I^2 = 72% indicates the use of fixed effect model over insignificant heterogeneity. There was significant difference in the production of IFN-λ by PBECs between the experimental group (asthmatics) and the control group (health controls) after *ex vivo* RV infection, with $p < 0.0001$ ($p < 0.05$) at 95% CI.

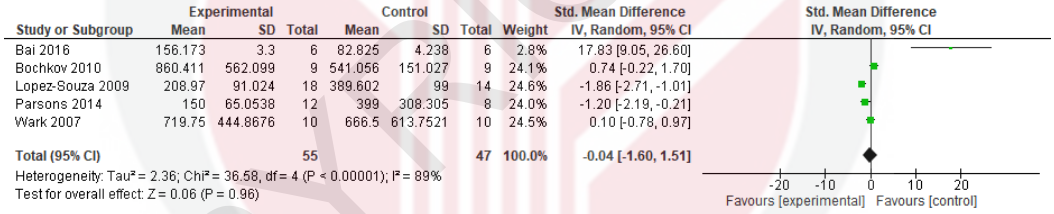

Figure 4.3.3. Forest plot of the meta-analysis of IL-6 from 5 studies with RV infection on *ex vivo* PBEC model. I^2 = 89% indicates the presence of significant heterogeneity, and random effect model was used. The figure showed that IL-6 production did not differ significantly between asthmatics and healthy controls following RV infection, with $p = 0.98$ at 95% CI.

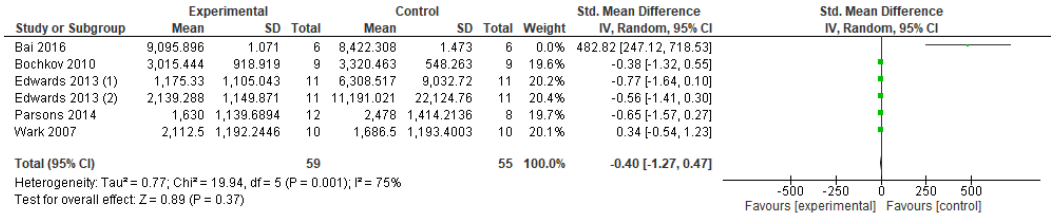

Figure 4.3.4. Forest plot of the meta-analysis of IL-8 from 6 studies with RV infection on *ex vivo* PBEC model. I^2 = 75% indicates the presence of significant heterogeneity, and random effect model was used. The plot showed that IL-8 production between asthmatics and healthy controls following RV infection was not significantly different, with p = 0.37 at 95% CI.

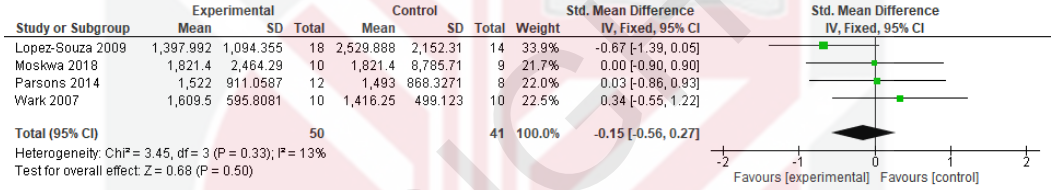

Figure 4.3.5. Forest plot of the meta-analysis of IP-10 from 4 studies with RV infection on *ex vivo* PBEC model. I^2 = 13% indicates a stronger homogeneity between the studies, and fixed effect model was used. The plot showed that IP-10 production did not differ significantly between asthmatics and healthy controls following RV infection, with p = 0.50 at 95% CI.

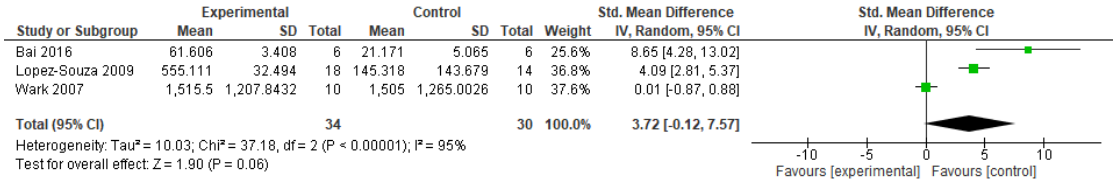

Figure 4.3.6. Forest plot of the meta-analysis of RANTES from 3 studies with RV infection on *ex vivo* PBEC model. I^2 = 95% indicates the presence of significant heterogeneity, and random effect model was used. The plot did not show a significant difference in RANTES production between asthmatics and healthy controls following RV infection, with p = 0.06 at 95% CI.

4.3.2 *Ex vivo* PBMC Studies

Figure 4.3.7 and Figure 4.3.8 showed the forest plots of meta-analysis of cytokines from *ex vivo* PBMC studies. A total of 2 cytokines were analysed under this category, namely IFN-α and IP-10. The cytokine levels were not significantly different between asthmatics and healthy controls after RV infection.

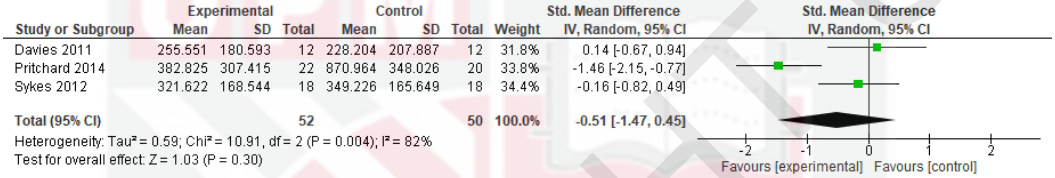

Figure 4.3.7 Forest plot of the meta-analysis of IFN-α from 3 studies with RV infection on *ex vivo* PBMC model. I^2 = 82% indicates a significant heterogeneity between the studies, and thus random effect model was used. The plot showed that IFN-α production by PCMCs did not differ significantly between asthmatics and healthy controls following RV infection, with p = 0.30 at 95% CI.

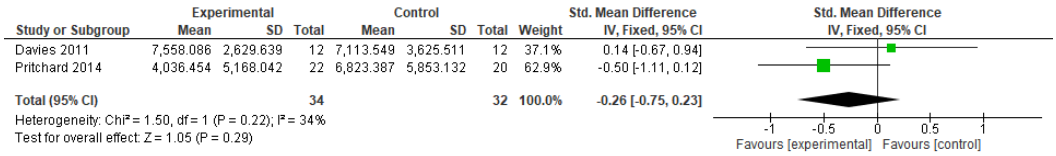

Figure 4.3.8. Forest plot of the meta-analysis of IP-10 from 2 studies with RV infection on *ex vivo* PBEC model. I^2 = 34% indicates a stronger homogeneity between the studies, and fixed effect model was used. The plot did not show a significant difference in IP-10 production by PBMCs between asthmatics and healthy controls following RV infection, with p = 0.29 at 95% CI.

4.3.3 Human Experimental Studies

Figure 4.3.9 - Figure 4.3.12 showed the forest plots of meta-analysis of cytokines from human experimental studies. Baseline and post-infection levels of IL-8 and IL-15 were compared. Post-infection IL-8 levels after experimental RV infection is significantly higher in bronchial fluids of asthmatics and baseline IL-15 levels before experimental RV infection were found to be significantly lower in bronchial fluids of asthmatics than healthy controls.

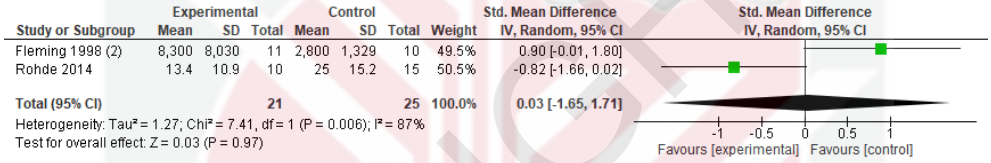

Figure 4.3.9. Forest plot of the meta-analysis of IL-8 at baseline level from 2 studies with experimental RV infection on human experimental model. $I^2 = 87\%$ indicates significant heterogeneity, and random effect model was used. The figure did not show a significant difference in baseline production of IL-8 between the asthmatics and healthy controls, with p = 0.97 at 95% CI.

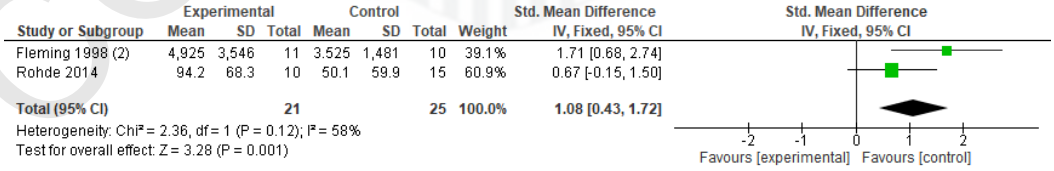

Figure 4.3.10. Forest plot of the meta-analysis of post-infection IL-8 levels from 2 studies with experimental RV infection on human experimental model. $I^2 = 58\%$ indicates insignificant heterogeneity, therefore fixed effect model was used. The figure shows a significant difference in IL-8 level in between the asthmatics and healthy controls after experimental RV infection, with p = 0.001 at 95% CI.

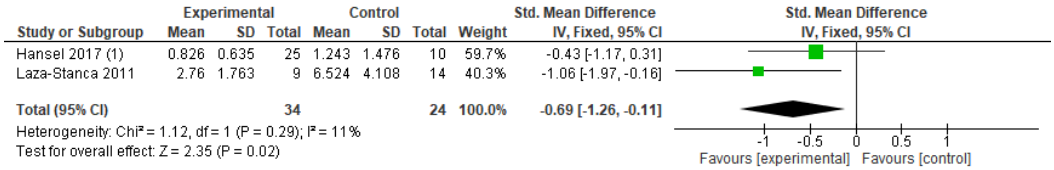

Figure 4.3.11. Forest plot of the meta-analysis of IL-15 at baseline level from 2 studies with experimental RV infection on human experimental model. I^2 = 11% indicates significant homogeneity, and fixed effect model was used. The figure shows a significant difference in baseline production of IL-15 between asthmatics and the healthy controls, with p = 0.02 at 95% CI.

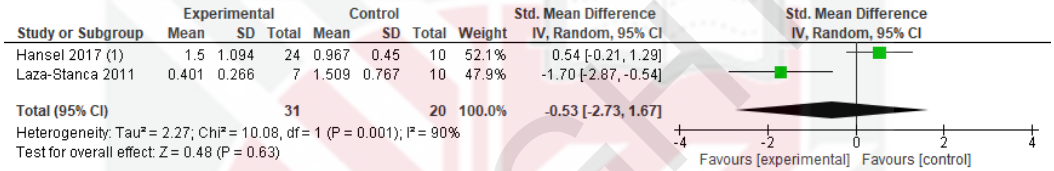

Figure 4.3.12. Forest plot of the meta-analysis of post infected IL-15 production from 2 studies with experimental RV infection on human experimental model. I^2 = 90% indicated significant heterogeneity, therefore random effect model was used. The figure did not show significant difference in IL-15 level in between the asthmatics and the healthy controls after experimental RV infection, with p = 0.63 at 95% CI.

4.4 Summary of Meta-Analysis

Table 4.3 shows the compilations of standard mean difference, p-value, and 95% confidence interval (CI) value of all the cytokines in both *ex vivo* and human experimental studies. The data were obtained from the results of meta-analysis using the software RevMan 5.3. $p < 0.05$ is considered significant. Cytokines that showed significant standard mean difference with p-values less than 0.05 were considered as significantly different in expression between asthmatics and the healthy control. This highlighted the potential of the cytokines as biomarkers for the identification and treatment of RV-induced asthma exacerbations. The potential cytokines are IFN-β (-0.52, $p = 0.008$) and IFN-λ (-0.81, $p < 0.0001$) in *ex vivo* PBEC studies, as well as post-infected IL-8 (1.08, $p < 0.001$) and baseline IL-15 (-0.69, $p = 0.02$) in human experimental studies. Significant IFN-β and IFN-λ results in *ex vivo* PBEC studies indicate a lower production of the said cytokines in asthmatic groups. Significant post-infection IL-8 in human experimental group indicates a higher IL-8 level production in the asthmatic groups, while significant baseline IL-15 levels indicates a significant decrease in production in asthmatic groups.

Table 4.3. Summary table of the findings of meta-analysis of cytokines in *ex vivo* and human experimental studies.

Cytokine	Number of studies	Heterogeneity (%)	Effect size		95% CI	
			Standard mean difference	p-value	Lower value	Upper value
***Ex vivo* PBEC**						
IFN-β	7	69%	-0.52	0.008	-0.90	-0.13
IFN-λ	6	72%	-0.81	<0.0001	-1.20	-0.41
IL-6	5	89%	-0.04	0.96	-1.60	1.51
IL-8	6	75%	-0.40	0.37	-1.27	0.47
IP-10	4	13%	-0.15	0.50	-0.56	0.27
RANTES	3	95%	3.72	0.06	-0.12	7.57
***Ex vivo* PBMC**						
IFN-α	3	82%	-0.51	0.30	-1.47	0.45
IP-10	2	34%	-0.26	0.29	-0.75	0.23
Human Experimental						
IL-8 (baseline)	2	87%	0.03	0.97	-1.65	1.71
IL-8 (post-infection)	2	58%	1.08	0.001	0.43	1.72
IL-15 (baseline)	2	11%	-0.69	0.02	-1.26	-0.11
IL-15 (post-infection)	2	90%	-0.53	0.63	-2.73	1.67

4.5 Publication Bias Assessment

Publication bias assessment was performed to estimate the overall effects from significant results and non-significant conclusions that might be underrepresented in publications to rule out bias in reporting only statistically positive results, especially in smaller studies (Sun et al., 2018). The publication bias for each meta-analysis was done by assessing the presence of asymmetry of the funnel plot, Egger's regression test, Begg's test, Rosenthal's fail-safe N, and Trim and Fill test. The assessment was performed with Meta Essential Microsoft Office Workbook. Publication bias is present when there is an asymmetry in the funnel plot with significant p-value ($p < 0.05$) in Egger's regression test and Begg's test.

4.5.1 *Ex vivo* PBEC

IFN-β

Figure 4.4.1. Funnel plot assessment of publication bias for IFN-β in *ex vivo* PBEC studies. Asymmetrical funnel plot indicates the presence of publication bias due to small study size.

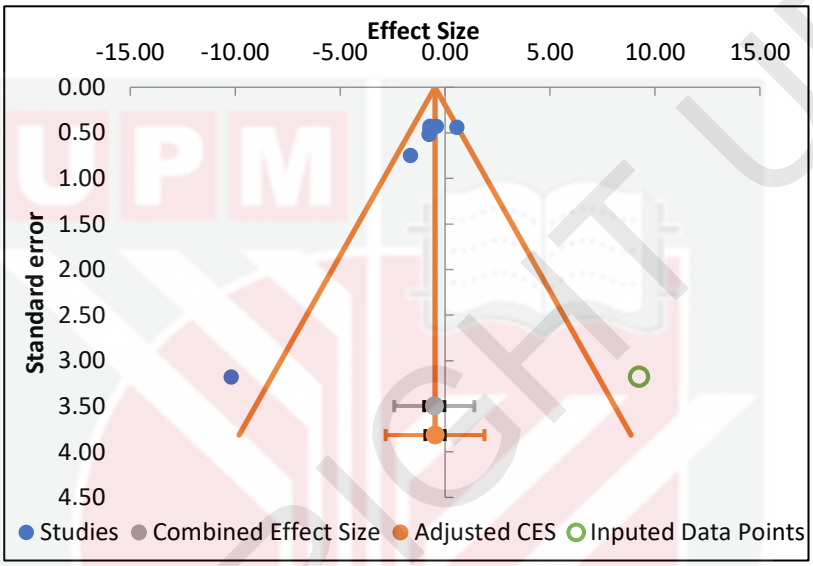

Table 4.4.1. Publication bias assessment for meta-analysis of *ex vivo* PBEC IFN-β. Egger's regression test and Begg's test were significant ($p < 0.05$) with presence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated an additional 29 zero effect size studies will nullify the statistical significance of the meta-analysis. Trim and fill method suggested 1 additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is present.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
***Ex vivo* PBEC IFN-β**	present	$p < 0.05$	$p < 0.05$	29	1	Publication bias: present

IFN-λ

Figure 4.4.2. Funnel plot assessment of publication bias for IFN-λ in *ex vivo* PBEC studies. The study size is small; however, the funnel plot does not show asymmetry, indicating the absence of publication bias.

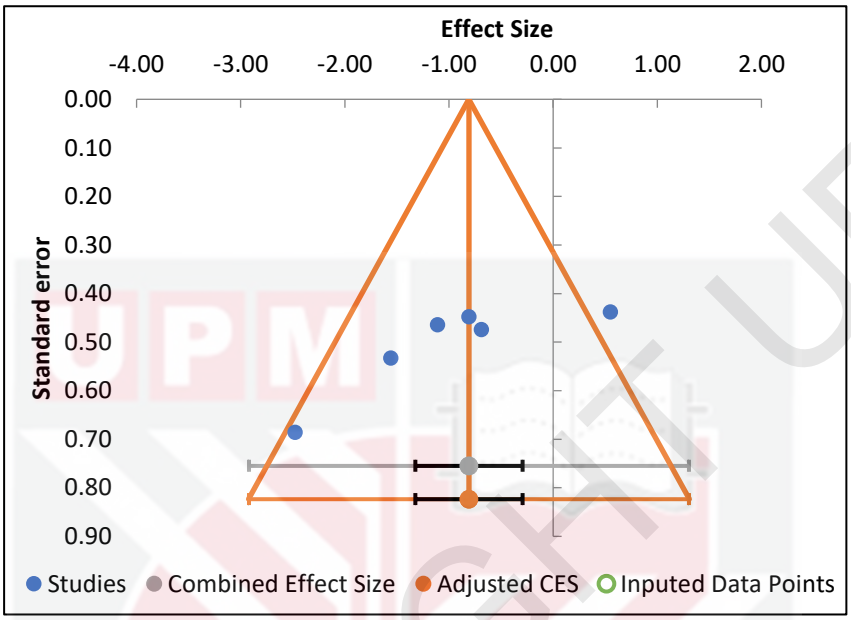

Table 4.4.2. Publication bias assessment for meta-analysis of *ex vivo* PBEC IFN-λ. Egger's regression test was insignificant ($p < 0.05$) while Begg's test was significant ($p < 0.05$) despite the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated an additional 38 zero effect size studies will nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias status is unclear.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
***Ex vivo* PBEC IFN-λ**	Absent	$p > 0.05$	$p < 0.05$	N = 38	0	Publication bias: unclear

IL-6

Figure 4.4.3. Funnel plot assessment of publication bias for IL-6 in *ex vivo* PBEC studies. The funnel plot does not show asymmetry, indicating the absence of publication bias.

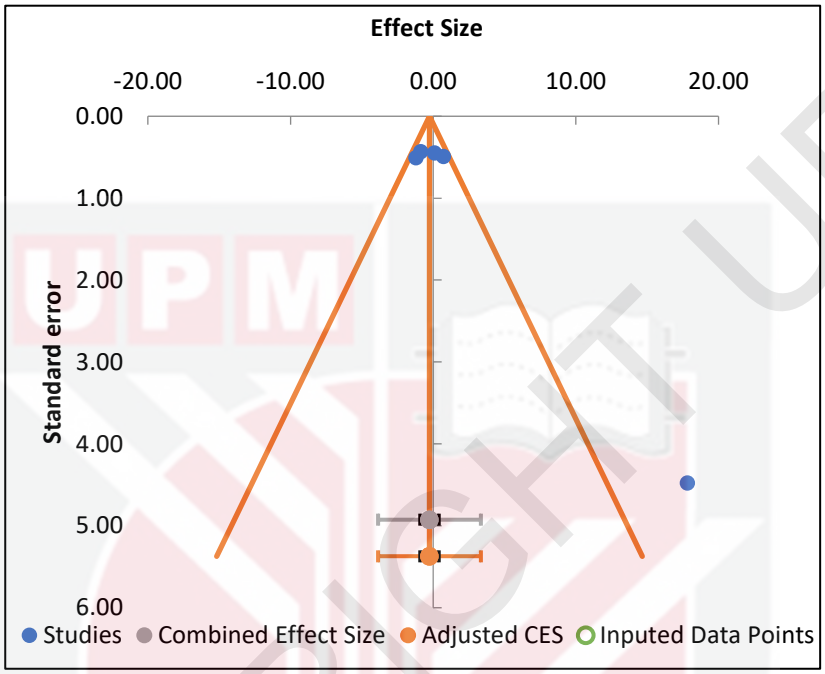

Table 4.4.3. Publication bias assessment for meta-analysis of *ex vivo* PBEC IL-6. Egger's regression test and Begg's test were insignificant ($p > 0.05$) with absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated no additional zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is absent.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
***Ex vivo* PBEC IL-6**	Absent	$p > 0.05$	$p > 0.05$	0	0	Publication bias: no

IL-8

Figure 4.4.4. Funnel plot assessment of publication bias for IL-8 in *ex vivo* PBEC studies. Despite the small study size, the funnel plot does not show asymmetry, indicating the absence of publication bias.

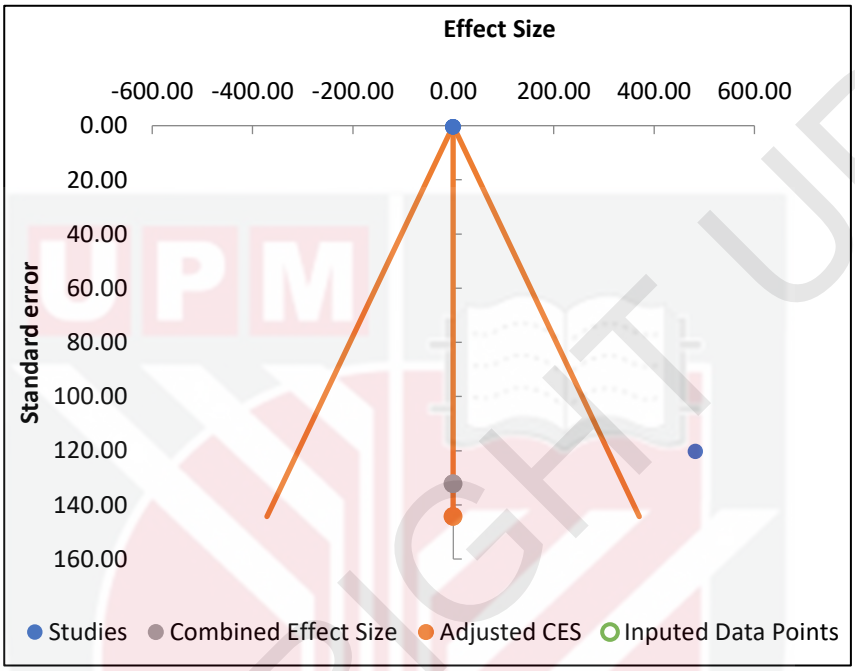

Table 4.4.4. Publication bias assessment for meta-analysis of *ex vivo* PBEC IL-8. Egger's regression test was significant ($p < 0.05$) while Begg's test was insignificant ($p > 0.05$) despite the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated no additional zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is unclear.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
***Ex vivo* PBEC IL-8**	Absent	$p < 0.05$	$p > 0.05$	0	0	Publication bias: unclear

IP-10

Figure 4.4.5. Funnel plot assessment of publication bias for IP-10 in *ex vivo* PBEC studies. No asymmetry in the funnel plot was observed, indicating the absence of publication bias.

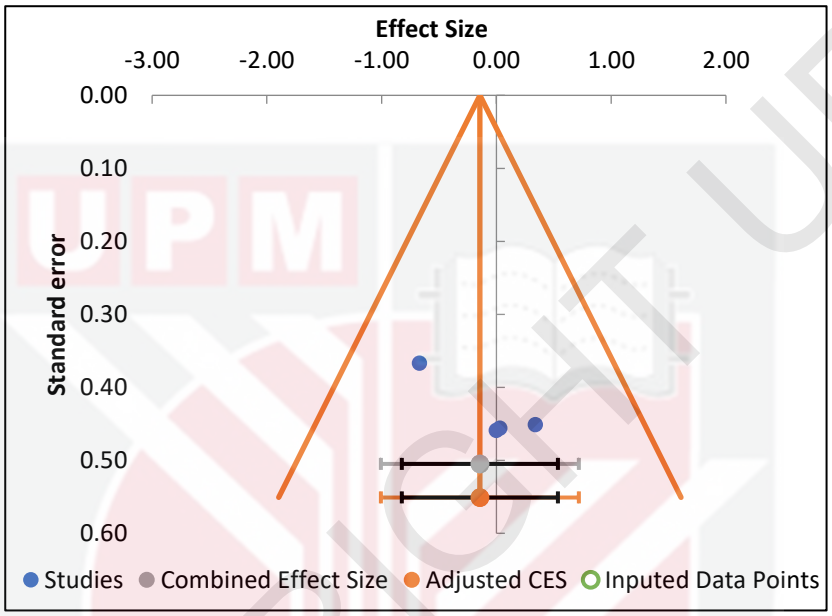

Table 4.4.5. Publication bias assessment for meta-analysis of *ex vivo* PBEC IP-10. Egger's regression test and Begg's test were insignificant ($p > 0.05$) with the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated no additional zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is unclear.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
***Ex vivo* PBEC IP10**	Present	$p > 0.05$	$p > 0.05$	0	0	Publication bias: no

RANTES

Figure 4.4.6. Funnel plot assessment of publication bias for RANTES in *ex vivo* PBEC studies. Asymmetry of the funnel plot indicates the presence of publication bias due to small study size.

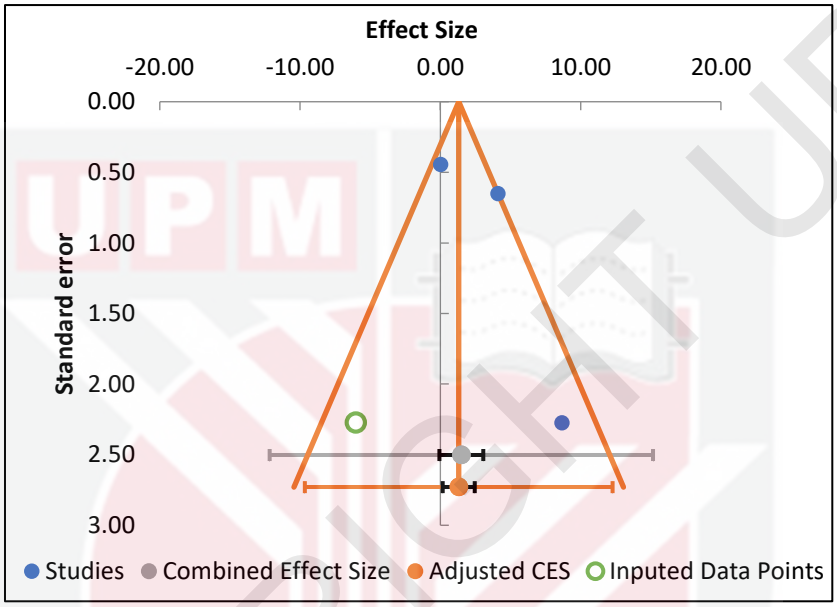

Table 4.4.6. Publication bias assessment for meta-analysis of *ex vivo* PBEC RANTES. Egger's regression test and Begg's test were insignificant ($p > 0.05$) despite the presence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated an additional 35 zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested 1 additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is unclear.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
***Ex vivo* PBEC RANTES**	Present	$p > 0.05$	$p > 0.05$	35	1	Publication bias: unclear

4.5.2 *Ex vivo* PBMC

IFN-α

Figure 4.4.7. Funnel plot assessment of publication bias for IFN-α in *ex vivo* PBMC studies. The plot did not show visible asymmetry despite the small study number, indicating the absence of publication bias.

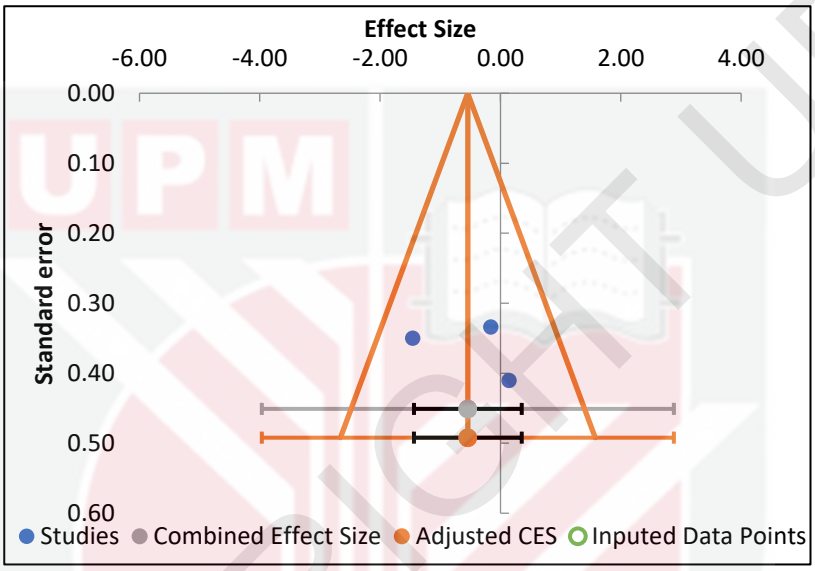

Table 4.4.7. Publication bias assessment for meta-analysis of *ex vivo* PBMC IFN-α. Egger's regression test and Begg's test were insignificant ($p > 0.05$) together with the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated an additional 4 zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is absent.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
***Ex vivo* PBMC IFN-α**	Absent	$p > 0.05$	$p > 0.05$	4	0	Publication bias: no

IP-10

Figure 4.4.8. Funnel plot assessment of publication bias for IP-10 in *ex vivo* PBMC studies. No asymmetry of the funnel plot was observed despite the small study size, indicating the absence of publication bias.

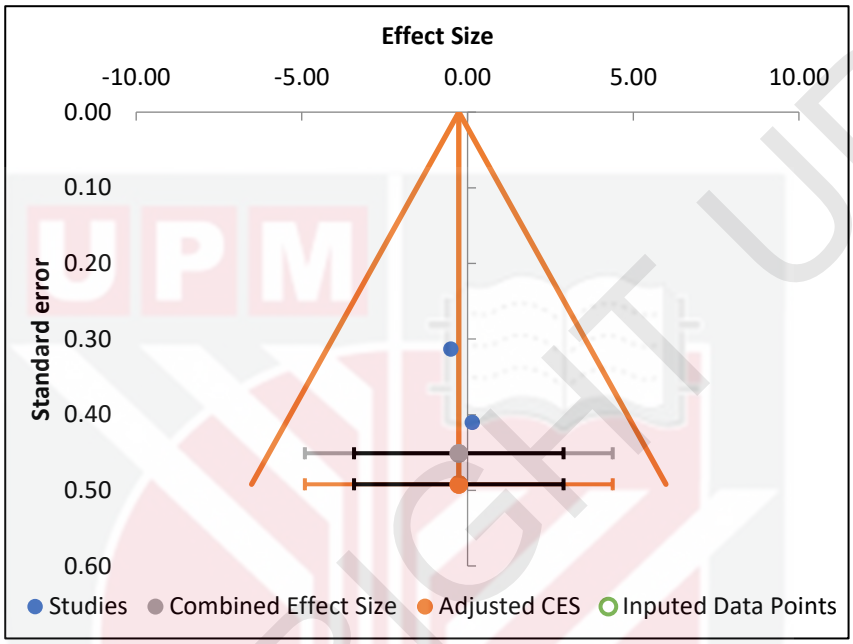

Table 4.4.8. Publication bias assessment for meta-analysis of *ex vivo* PBMC IP-10. Egger's regression test was unavailable due to insufficient study number, while Begg's test was insignificant ($p > 0.05$) together with the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated no additional zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is absent.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
***Ex vivo* PBMC IP10**	Absent	NA	$p > 0.05$	0	0	Publication bias: no

4.5.3 Human Experimental (Lower Respiratory Tract)

IL-8 (Baseline)

Figure 4.4.9. Funnel plot assessment of publication bias for baseline levels of IL-8 in human experimental studies. No asymmetry of the funnel plot was observed despite the small study size. This indicated the absence of publication bias.

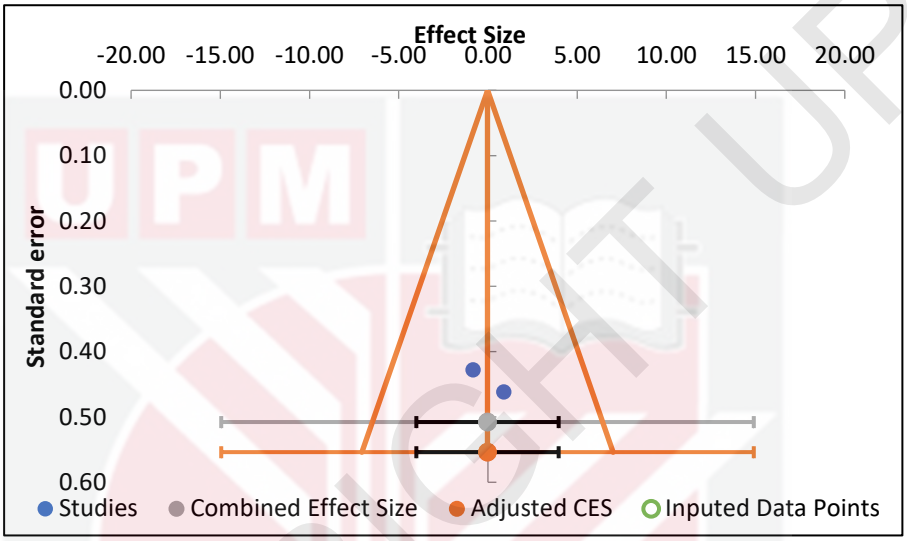

Table 4.4.9. Publication bias assessment for meta-analysis of baseline levels of IL-8 in human experimental studies. Egger's regression test was unavailable due to insufficient study number, while Begg's test was insignificant ($p > 0.05$) together with the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated no additional zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is absent.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
Human experimental IL-8 baseline	Absent	NA	$p > 0.05$	0	0	Publication bias: no

IL-8 (Post Infection)

Figure 4.4.10. Funnel plot assessment of publication bias for post-infection levels of IL-8 in human experimental studies. No asymmetry was observed in the funnel plot despite the small study size, indicative of absence of publication bias.

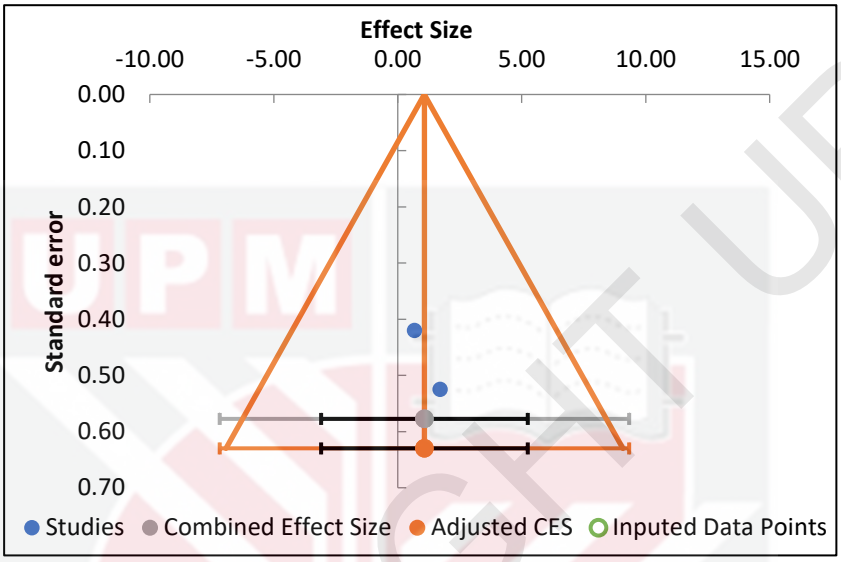

Table 4.4.10. Publication bias assessment for meta-analysis of post-infection levels of IL-8 in human experimental studies. Egger's regression test was unavailable due to insufficient study number, while Begg's test was insignificant ($p > 0.05$) together with the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated 7 additional zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is absent.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
Human experimental IL-8 post infection	Absent	NA	$p > 0.05$	7	0	Publication bias: no

IL-15 (Baseline)

Figure 4.4.11. Funnel plot assessment of publication bias for baseline levels of IL-15 in human experimental studies. No asymmetry was observed in the funnel plot despite the small study size. This indicated the absence of publication bias.

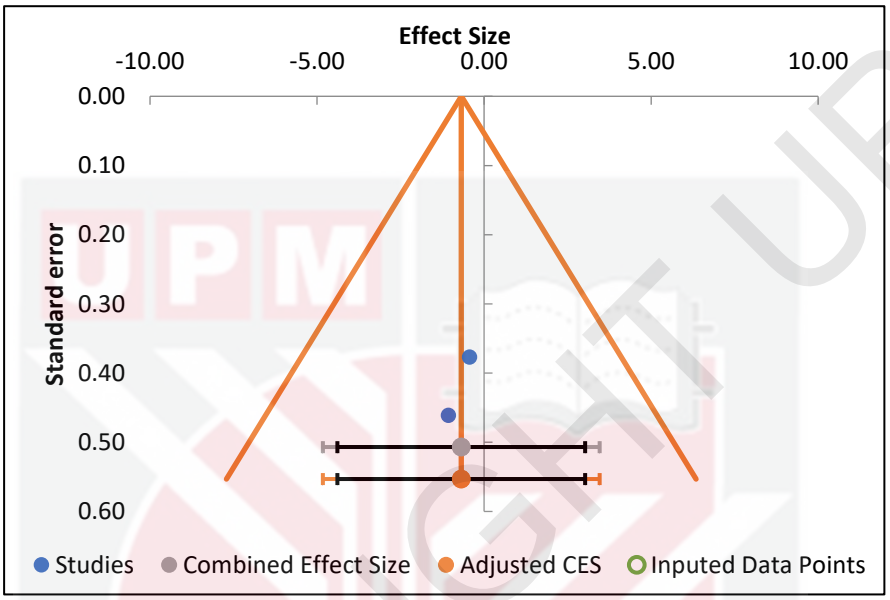

Table 4.4.11. Publication bias assessment for meta-analysis of baseline levels of IL-15 in human experimental studies. Egger's regression test was unavailable due to insufficient study number, while Begg's test was insignificant ($p > 0.05$) together with the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated 2 additional zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is absent.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
Human Experimental IL-15 baseline	Absent	NA	$p > 0.05$	2	0	Publication bias: no

IL-15 (Post Infection)

Figure 4.4.12. Funnel plot assessment of publication bias for post-infection levels of IL-15 in human experimental studies. The funnel plot did not show visible asymmetry, indicating the absence of publication bias.

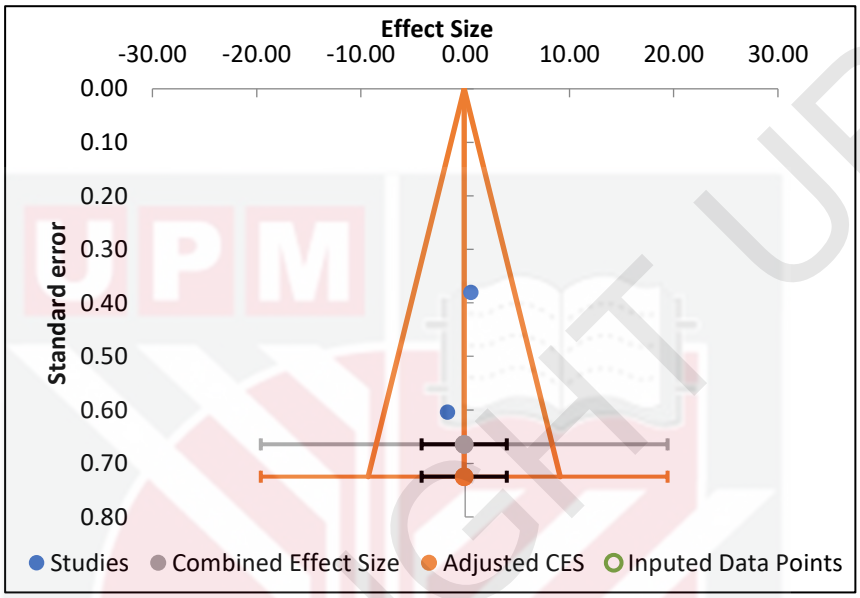

Table 4.4.12. Publication bias assessment for meta-analysis of post-infection levels of IL-15 in human experimental studies. Egger's regression test was unavailable due to insufficient study number, while Begg's test was insignificant ($p > 0.05$) together with the absence of asymmetry in the funnel plot. Rosenthal's fail-safe N indicated no additional zero effect size studies will be needed to nullify the statistical significance of the meta-analysis. Trim and fill method suggested no additional study needed to be imputed to correct the funnel plot from asymmetry. Publication bias is absent.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion
Human experimental IL-15 post infection	Absent	NA	$p > 0.05$	0	0	Publication bias: no

Table 4.5. Summary of publication bias across all meta-analyses of cytokines in *ex vivo* and human experimental models.

Type of Study	Asymmetry of Funnel Plot	Egger's regression test	Begg's test	Rosenthal's fail-safe N	Trim and fill (imputed data points)	Conclusion	Remarks
***Ex vivo* PBEC IFN-β**	Present	$p < 0.05$	$p < 0.05$	29	1	Publication bias: yes	
***Ex vivo* PBEC IFN-λ**	Absent	$p > 0.05$	$p < 0.05$	38	0	Publication bias: unclear	
***Ex vivo* PBEC IL-6**	Absent	$p > 0.05$	$p > 0.05$	0	0	Publication bias: no	
***Ex vivo* PBEC IL-8**	Absent	$p < 0.05$	$p > 0.05$	0	0	Publication bias: unclear	
***Ex vivo* PBEC IP10**	Absent	$p > 0.05$	$p > 0.05$	0	0	Publication bias: no	
***Ex vivo* PBEC RANTES**	Present	$p > 0.05$	$p > 0.05$	35	1	Publication bias: unclear	
***Ex vivo* PBMC IFN-α**	Absent	$p > 0.05$	$p > 0.05$	4	0	Publication bias: no	
***Ex vivo* PBMC IP10**	Absent	NA	$p > 0.05$	0	0	Publication bias: no	Egger's test unavailable due to two studies compared only
Human experimental IL-8 baseline	Absent	NA	$p > 0.05$	0	0	Publication bias: no	Egger's test unavailable due to two studies compared only
Human experimental IL-8 post infection	Absent	NA	$p > 0.05$	7	0	Publication bias: no	Egger's test unavailable due to two studies compared only
Human Experimental IL-15 baseline	Absent	NA	$p > 0.05$	2	0	Publication bias: no	Egger's test unavailable due to two studies compared only
Human experimental IL-15 post infection	Absent	NA	$p > 0.05$	0	0	Publication bias: no	Egger's test unavailable due to two studies compared only

CHAPTER 5

DISCUSSION

This study aims to uncover which cytokine will be significantly different in production between asthmatics and healthy controls upon RV infection. Previous studies agreed on the "dysregulated cytokine production" concept as the main driving force of asthma exacerbations. However, many researchers did not come to a same conclusion on which cytokine exactly was elevated or deficient in production. Therefore, it is hard to pinpoint a cytokine that is significantly different in production by asthmatics following RV infection, which researchers can collectively agree upon. As such, meta-analysis was performed to identify key cytokine production during RV-induced asthma exacerbations by computing the overall effect estimate of previous studies under each cytokine category for each experimental group.

The meta-analysis began by identifying articles from databases. Then, selection criteria were applied to filter out unqualified records. As a consideration to the nature of RV infection and site of cytokine production, the meta-analysis was categorized into three experimental groups, which are (1) *ex vivo* PBEC, (2) *ex vivo* PBMC and (3) human experimental studies. Detailed characterization of

each study was performed to identify variations in study designs which can be potential limitations to the meta-analysis.

Previous findings on the mode of RV infection uncovered its major route of infection: through binding of ICAM-1 receptor located on cells surfaces of PBECs and PBMCs (Papadopoulus et al., 2004). Therefore, the meta-analysis compared cytokine profile from *ex vivo* PBEC and PBMC models. From Table 4.1, 12 papers out of 19 qualified papers for meta-analysis were *ex vivo* PBEC studies. This further highlighted the role of PBEC as a major target of RV infection and site of asthmatic cytokine production. RV is also a major pathogen of the lower respiratory tract that can potentiate an asthma exacerbation (Jacobs et al., 2013). This explain why we only select findings which obtained samples from lower respiratory tracts (e.g. bronchoalveolar lavage, bronchial mucosal lining, sputum) in human experimental studies, which could have greater pathological and cytokine production significance.

The results from the meta-analysis were represented in the schematic diagram Figure 4.1. and forest plots in Figure 4.3.1 to Figure 4.3.12. Meta-analysis for each cytokine was performed only when there are two or more findings reporting under the same experimental model. For each cytokine, the overall effect was calculated by measuring the differences between the sum of effects of both groups. From the forest plots, we can observe that studies with greater sample size have greater weights as represented by the boxes. This represents the strength of each study that contributes toward the meta-analysis.

5.1 *Ex vivo* PBEC IFN-β and IFN-λ

Overall, the key cytokines identified from the meta-analysis are *ex vivo* PBEC IFN-β and IFN-λ, human experimental post-infected IL-8 and IL-15. IFN-β and IFN-λ are part of the innate antiviral immune response. IFNs are needed to signal RV infected cells to undergo apoptosis, a reduced IFN production increases RV propagation, cytopathic tissue death and encourages Th2 cytokine-mediated inflammation which can cause asthmatic flare ups (Cakebread et al., 2011; Grainge and Davies, 2013). Meta-analysis of IFN-β and IFN-λ both revealed a significant deficient production by asthmatics PBECs *ex vivo* when challenged by RV, as shown in Figure 4.3.1 and 4.3.2. Since asthmatic PBECs are structurally and physiologically remodelled, IFN-β and IFN-λ production could also be greatly impaired. This is further confirmed by reports of severe asthmatics derived BECs being unable to produce sufficient IFN-β and IFN-λ after exposure to RVs (Wark et al., 2005; Contoli et al., 2006). Therefore, IFN-β and IFN-λ deficiency could be the first hallmarks of impaired immune response in asthmatics towards RV infections.

5.2 Human Experimental IL-8 and IL-15

IL-8 are Th2 cytokines responsible for neutrophilic infiltrations. By acting as a chemoattractant to recruit neutrophils and activated eosinophils to site of RV infections, they promote further inflammation in the pre-existing hypersensitive airway (Steinke and Borish, 2016). This causes mucous hypersecretion and

bronchial obstruction that will lead to asthma exacerbations (Gern and Busse, 1999, Gern et al., 2000). IL-8 could also lead to asthma exacerbations by increasing severity of cold, which is a major risk factor for hypersensitivity in asthmatics (Grünberg et al., 1997). From the meta-analysis, we found that asthmatics had significantly higher IL-8 levels following RV infection in human experimental models as shown in Figure 4.3.1. Synchronous with previous findings, increased IL-8 production in asthmatics may increase their risk of asthma attacks even in normal colds caused by RV infections. This could be a key cytokine finding to separate RV-induced asthma exacerbation episodes from normal cold immune responses.

IL-15 on the other hand, is a Th1 cytokine that works with IFNs to mount innate immune response and adaptive antiviral immune response (Carson et al., 1995). IL-15 recruits natural killer (NK) cells and CD8+ memory T cells to remove microbial pathogens. Past studies also showed that IL-15 levels were proportional to Type 1 IFN-α/β release, while being inversely proportional to viral load and airway hyperresponsiveness (Laza-Stanca et al., 2011). Meta-analysis result of baseline IL-15 in asthmatics were shown to be lower than normal healthy individuals. This finding could be related to significantly lowered IFN-β production by asthmatics upon RV infection detected in Figure 4.3.1. Asthmatics' inability to mount an effective clearance response of RV may lie in the defective production of IFNs and IL-15.

5.3 Limitations

Publication bias is a situation where positive and favourable results received more publications than negative and unfavourable results. This scenario will create a false impression of the magnitude of the actual effect (Dickersin and Min, 1993). Since meta-analysis is a process of critical assessment of previously published data, they are subjected to this unwanted effect of bias. Meta-analysis relies heavily on the sample sizes and availability of comparison studies in order to generate a precise evaluation of overall effect in a particular finding (Møller and Myles, 2016). As such, a single paper reporting multiple findings of different infection model could unknowingly skew the results due to similar study subjects. Similar subjects also reduced heterogeneity, which is favoured in meta-analysis. This might have affected the outcome of the particular research due to similar response towards an experimental intervention, and this may not represent the response of the whole population. This could have affected the meta-analysis outcome of IFN-β, where a single paper by Wark et al. (2005) reported 3 findings by using similar study subjects but with different RV serotypes as intervention. This might be the reason for the presence of publication bias indicated in the meta-analysis of IFN-β.

In a usual detection of publication bias, one will examine the funnel plot for presence of asymmetry, then proceed to check for the p-values of Egger's regression test and Begg's test. A conclusive detection of publication bias will find (1) asymmetry of funnel plot, (2) significant p-value for Egger's regression test

and Begg's test ($p < 0.05$). Egger's regression test of IFN-λ detected significant presence of bias after regressing the effect size against the precision of the studies ($p < 0.05$). Begg's test did not detect any presence of bias, but when a meta-analysis has less than 25 studies, power of this test will grow considerably weak (Kale and Nirpharake, 2017). Both Egger's test and Begg's test has low detection power if the number of studies included in meta-analysis is low, hence we should interpret small meta-analyses with caution (Lin and Chu, 2018). Since *ex vivo* PBEC IFN-λ has conflicting statistical tests, the publication bias for this meta-analysis is rather unclear, which limits the statistical significance of the meta-analysis.

As mentioned above, meta-analysis relied heavily on sample sizes. In our meta-analysis where only minimal number of findings with small sample size were primarily found, publication bias could happen easily because smaller sample sizes show greater extremes of treatment effects (Turner et al., 2013). This is evident in the meta-analysis of human experimental IL-8 and IL-15 levels (Figure 4.4.10 and Figure 4.4.11). Each data is distributed at the lower part of the funnel plot, indicating the lack of precision in each study due to small sample size. Egger's regression test is also unavailable for these two cytokines because of limitations in the study number. Although Rosenthal's fail-safe N for IL-8 and IL-15 is measurable, however a rather small number of negative findings are sufficient to nullify the significance of the combined effects of these two meta-analyses, which indicates the presence of publication bias (Kale and Nirpharake, 2017). These findings may limit the statistical significance of IL-8 and IL-15 as a key finding in RV-induced asthma exacerbations.

The use of different RV serotypes in experimental infections may potentially affect the severity of asthma exacerbations. While studies showed that major RV subtypes infect cells through ICAM-1 binding, minor subtypes of RV can also infiltrate cells through bindings with low-density lipoprotein receptors (LDLPRs) on these cell surfaces (Blaas and Fuchs, 2016). RV-A and RV-C subtypes can elicit a stronger immune response than RV-B subtypes (Jackson et al., 2016; Jartti and Gern, 2017). Even so, the downstream pathway of RV pathogenesis and IFN productions were reported to be independent of receptor types (Stone and Miller, 2015; Khaitov et al., 2009). Therefore, data from papers which experimented with multiple RV serotypes in their studies were also included in the meta-analysis as independent findings by assuming all RV serotypes will elicit similar level of immune response. However, further studies are needed to determine if different RV serotypes will indeed influence cytokine expression in asthmatics.

Another limitation of this meta-analysis is the atopy and medication status of asthmatics. There are reports of increased risk for asthma exacerbations among atopic individuals when tested both *in vivo* and *in vitro* (Papadopoulous et al., 2004). It is also common for asthmatic individuals to be on medication such as corticosteroids or budesonide. Although not capable of fully suppressing viral induced inflammations, corticosteroids may grant asthmatics certain degree of immunosuppression during viral infections (Oliver et al., 2014). However, due to limited number of studies identified, we included all asthmatic subjects regardless of atopy and medication status in the meta-analysis. This might be an important factor to inconsistent findings of cytokine production by asthmatics upon RV

infection. For greater statistical significance, meta-analysis should employ a greater degree of critical appraisal of every aspect of study intervention to ensure fewer possible variances due to experimental design differences.

CHAPTER 6

CONCLUSION

Overall, this meta-analysis had identified four cytokines, IFN-β, IFN-λ, IL-8 and IL15 as potential markers of dysregulated cytokine production in asthmatics upon RV infection. Significant deficient IFN-β and IFN-λ production in asthmatic PBECs in *ex vivo* studies upon RV infection, increased post-infection IL-8 production and lowered baseline IL-15 level in asthmatic human subjects may increase the risk of RV-induced asthma exacerbation. However, publication bias is the limitation for the meta-analysis findings of IFN-β and IFN-λ. Meta-analysis of IL-8 and IL-15 is also inconclusive due to limited study size and unclear publication bias. To overcome the shortcoming of publication bias, more studies with larger sample sizes are recommended to be included into each comparison group for better statistical significance, and more accurate estimation of publication bias. Even so, identification of these cytokines could be useful in giving better insights to design a targeted therapy for the alleviation of RV-induced exacerbations in asthmatics. Future studies on improving IFN productions and regulating Th1 and Th2 cytokine expression may help to prevent RV-induced asthma exacerbations.

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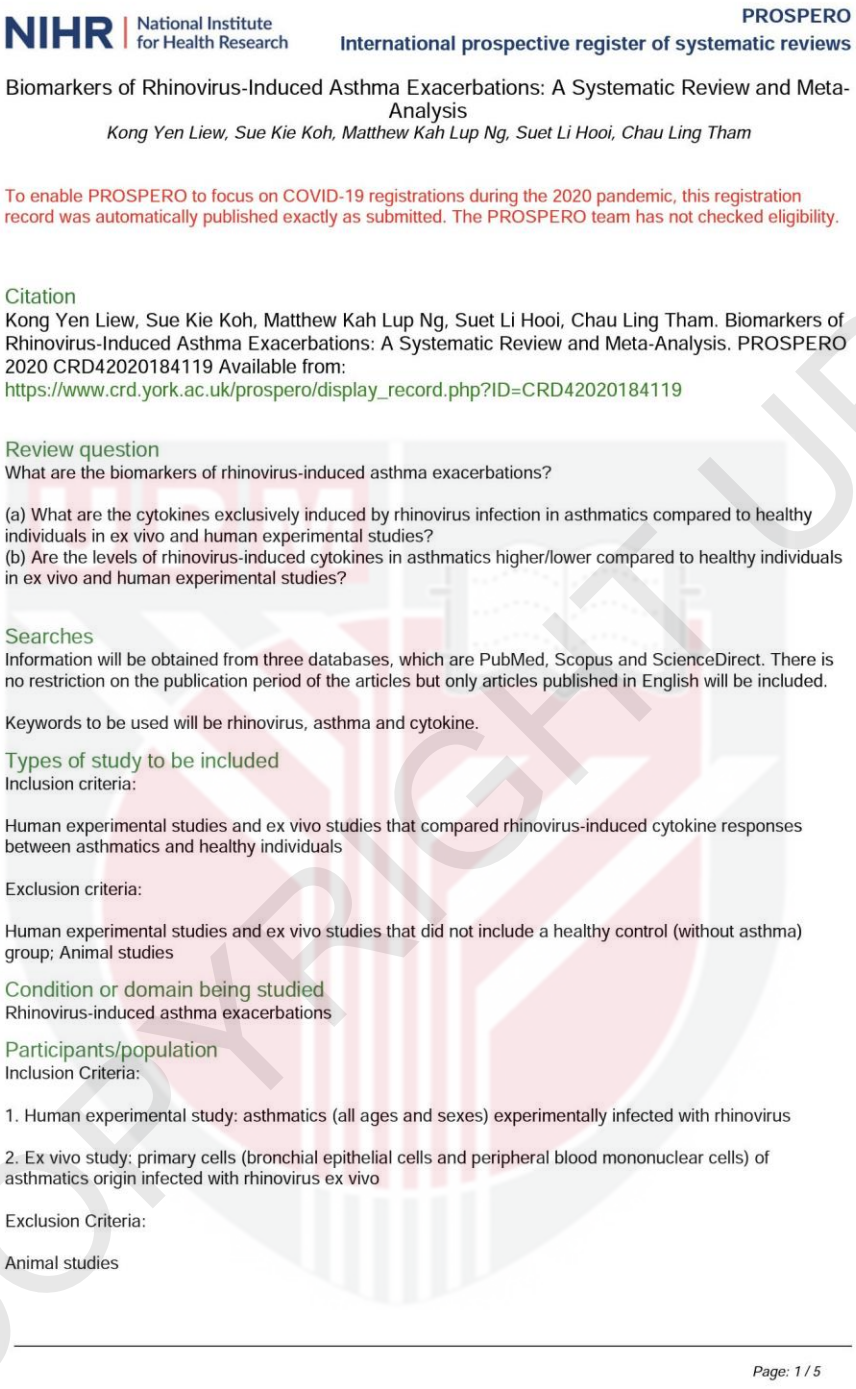
APPENDICES

Appendix A: Registration Form of International Prospective register of Systematic Reviews (PROSPERO)

PROSEPERO is a database for systematic review on various healthcare topics. It allows past findings of systematic review protocols to be recorded and kept as permanent record. One should register for PROSPERO when the systematic review is at the early protocol stage to avoid accidental duplication and to allow researchers to comparison with various research methods to avoid wastage of effort.



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PROSPERO
International prospective register of systematic reviews

Biomarkers of Rhinovirus-Induced Asthma Exacerbations: A Systematic Review and Meta-Analysis

Kong Yen Liew, Sue Kie Koh, Matthew Kah Lup Ng, Suet Li Hooi, Chau Ling Tham

To enable PROSPERO to focus on COVID-19 registrations during the 2020 pandemic, this registration record was automatically published exactly as submitted. The PROSPERO team has not checked eligibility.

Citation

Kong Yen Liew, Sue Kie Koh, Matthew Kah Lup Ng, Suet Li Hooi, Chau Ling Tham. Biomarkers of Rhinovirus-Induced Asthma Exacerbations: A Systematic Review and Meta-Analysis. PROSPERO 2020 CRD42020184119 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020184119

Review question

What are the biomarkers of rhinovirus-induced asthma exacerbations?

(a) What are the cytokines exclusively induced by rhinovirus infection in asthmatics compared to healthy individuals in ex vivo and human experimental studies?
(b) Are the levels of rhinovirus-induced cytokines in asthmatics higher/lower compared to healthy individuals in ex vivo and human experimental studies?

Searches

Information will be obtained from three databases, which are PubMed, Scopus and ScienceDirect. There is no restriction on the publication period of the articles but only articles published in English will be included.

Keywords to be used will be rhinovirus, asthma and cytokine.

Types of study to be included

Inclusion criteria:

Human experimental studies and ex vivo studies that compared rhinovirus-induced cytokine responses between asthmatics and healthy individuals

Exclusion criteria:

Human experimental studies and ex vivo studies that did not include a healthy control (without asthma) group; Animal studies

Condition or domain being studied

Rhinovirus-induced asthma exacerbations

Participants/population

Inclusion Criteria:

1. Human experimental study: asthmatics (all ages and sexes) experimentally infected with rhinovirus

2. Ex vivo study: primary cells (bronchial epithelial cells and peripheral blood mononuclear cells) of asthmatics origin infected with rhinovirus ex vivo

Exclusion Criteria:

Animal studies

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Figure 1: Page 1 of PROSPERO Registration Form.

Intervention(s), exposure(s)

Inclusion Criteria:

All species or subtypes of rhinoviruses in any dose

Exclusion Criteria:

Respiratory viruses other than rhinoviruses

Comparator(s)/control

Inclusion Criteria:

1. Human experimental study: healthy humans without asthma (all ages and sexes) experimentally infected with rhinovirus

2. Ex vivo study: primary cells (bronchial epithelial cells and peripheral blood mononuclear cells) originated from healthy humans without asthma which were infected with rhinovirus ex vivo

Exclusion Criteria:

None

Main outcome(s)

Human Experimental Study:

Concentration (pg/mL) of cytokines in biological samples such as sputum, bronchoalveolar lavage fluid and bronchial mucosal lining fluid

Ex Vivo Study:

Concentration (pg/mL) of cytokines in culture supernatants of primary cells (bronchial epithelial cells and peripheral blood mononuclear cells)

Measurement of the concentration of cytokines was done with enzyme-linked immunosorbent assay (ELISA) or other

immunoassays.

* Measures of effect

Human Experimental Study:

Concentration of cytokines induced by rhinovirus infection: the standardized mean difference between asthmatics and healthy individuals with 95% confidence interval (CI); the heterogeneity percentage

Ex Vivo Study:

Concentration of cytokines induced by rhinovirus infection: the standardized mean difference between cells originated from asthmatics and cells originated from healthy individuals with 95% confidence interval (CI); the heterogeneity percentage

Additional outcome(s)

None

* Measures of effect

None

Figure 2: Page 2 of PROSPERO Registration Form.

Data extraction (selection and coding)

Data Selection:

All articles obtained from the three databases using specific keywords will be organized using Microsoft Excel Spreadsheet according to their titles and duplicates of the same study will be removed. All the non-redundant articles will be independently screened by two reviewers for relevant studies according to the eligibility criteria. Full text of the remaining studies will then be screened again to further determine their relevancy. Disagreements between the two reviewers will be resolved by consensus and the reason for excluding the studies will be recorded.

Data Extraction:

1. For human experimental study, the following data will be extracted: participant demographics (age and sex) and their allergy status, sample size, rhinovirus (species, subtypes and dose for experimental infection), types of biological samples (sputum, bronchoalveolar lavage fluid or bronchial mucosal lining fluid), types and levels (mean and standard deviation) of cytokines measured, comparison of cytokine levels (including the significance level) between asthmatic and healthy control group and method of cytokines measurement.

2. For ex vivo study, the following data will be extracted: types of primary cells used (bronchial epithelial cells or peripheral blood mononuclear cells), donors (asthmatics or healthy individuals) and their demographics and allergy status, rhinovirus (species, subtypes and dose for ex vivo infection), types and levels (mean and standard deviation) of cytokines measured, comparison of cytokine levels (including the significance level) between asthmatic and healthy control group and method of cytokine measurement.

3. Methods of data extraction: for cytokines of which the concentration (pg/mL) was not given but presented in graphs instead, data will be extracted from the graphs using the software ImageJ version 150.

4. All the data extracted will be organized using Microsoft Excel Spreadsheet by the first reviewer and checked by the
second reviewer. Disagreements between the two reviewers will be resolved by discussion with the third reviewer.

Risk of bias (quality) assessment

Publication bias of each cytokine included in the meta-analysis will be assessed using Funnel plot, Begg's test, Egger's Regression test and Rosenthal's Fail-safe N.

Strategy for data synthesis

Systematic Review (Qualitative Synthesis):

The findings will be grouped into 3 categories, which are (1) human experimental study, (2) ex vivo bronchial epithelial cells study, and (3) ex vivo peripheral blood mononuclear cells study. The comparison of cytokine levels between asthmatic and healthy control group, whether significantly higher in asthmatic group, no significant difference between groups or significantly lower in asthmatic group, reported by each study will be recorded.

Meta-Analysis (Quantitative Synthesis):

Meta-analysis will be performed for the cytokine(s) which was reported in at least 2 studies in respective categories, which are (1) human experimental study, (2) ex vivo bronchial epithelial cells study, and (3) ex vivo peripheral blood mononuclear cells study.

For each cytokine, the levels (pg/mL) of both groups (asthmatic and healthy control) will be inserted into the software

Review Manager (RevMan) version 5.3. The standardized mean difference of cytokine levels between the two groups, including the 95% CI, for each study will be computed and the overall effect will be determined

Figure 3: Page 3 of PROSPERO Registration Form.

using a fixed-effect or random-effects statistical model. A fixed-effect model will be used if the heterogeneity between the studies (I^2) is less than 75% while a random-effects model will be used if I^2 is more than 75%. The heterogeneity between studies will be considered significant if I^2 is more than 75%.

Analysis of subgroups or subsets
No analysis of subgroups or subsets planned

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Type and method of review
Meta-analysis, Systematic review

Anticipated or actual start date
01 April 2020

Anticipated completion date
31 December 2020

Funding sources/sponsors
Fundamental Research Grant Scheme (FRGS) (FRGS/1/2018/SKK10/UPM/02/2) from Ministry of Education Malaysia

Grant number(s)
FRGS/1/2018/SKK10/UPM/02/2

Conflicts of interest

Language
English

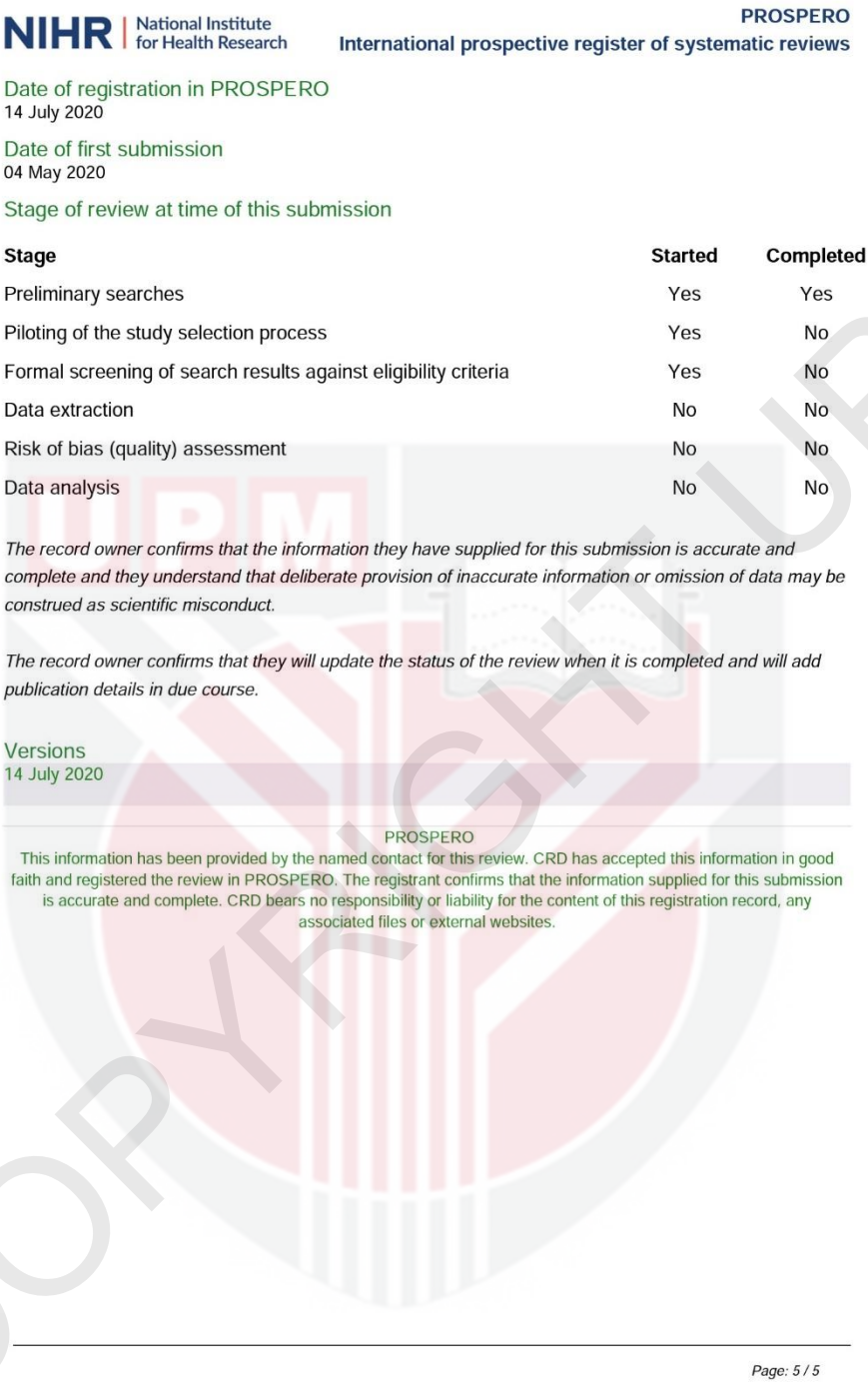
Country
Malaysia

Stage of review
Review Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
MeSH headings have not been applied to this record

Figure 4: Page 4 of PROSPERO Registration Form.



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PROSPERO
International prospective register of systematic reviews

Date of registration in PROSPERO
14 July 2020

Date of first submission
04 May 2020

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.

The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions
14 July 2020

PROSPERO
This information has been provided by the named contact for this review. CRD has accepted this information in good faith and registered the review in PROSPERO. The registrant confirms that the information supplied for this submission is accurate and complete. CRD bears no responsibility or liability for the content of this registration record, any associated files or external websites.

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Figure 5: Page 5 of PROSPERO Registration Form.

Appendix B: Meta-analysis

Articles for Full Text Screening

A search with the keywords “rhinovirus* AND asthma* AND (cytokine* OR chemokine* OR interferon* OR antiviral*)” through the databases yielded 2158 search results. After screening through the abstracts and titles according to the selection criteria, a total of 32 articles were found to be eligible for full length article screening. This screening process further determined whether these articles are suitable for the meta-analysis. A total of 19 studies were qualified, while the rest of the articles were rejected with reasons provided in Table 1.

Table 1: Details of 32 articles eligible for full length article screening.

***Ex vivo* PBEC studies**							
No.	**Title**	**Year**	**Comparison (Sample size)**	**RV**	**Cytokine/Chemokine**	**Remark**	**Acceptance**
1	Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus	2005	Atopic asthmatics (14) - GINA; Non-atopic healthy (10); Demo provided; Non-smokers; ICS and ICS naive	RV-16; 2 MOI	IFN-β (median-IQR), IL-6, RANTES (median-IQR-fold induction); 48 hours		Accepted
2	Role of deficient type III interferon-lambda production in asthma exacerbations	2006	Atopic asthmatics (9); Healthy (9); Demo provided; Non-smokers; w, w/o ICS	RV-16; 2 MOI	IFN-λ (should be mean-SD); 48 hours	Human Experimental + BAL cells	Accepted
3	IFN-γ–induced protein 10 is a novel biomarker of rhinovirus-induced asthma exacerbations	2007	Atopic asthmatics (10); Healthy (10); Demo provided; Non-smokers; w/o ICS	RV-16; 2 MOI	IL-6, IL-8, RANTES, IP-10 (box plot)	Clinical (Virus-induced Asthma) Serum Cytokines	Accepted
4	Diversity in the bronchial epithelial cell response to infection with different rhinovirus strains	2009	Atopic asthmatics (4); Non-atopic healthy (4); Demo provided; Non-smokers; w ICS	RV-1B; 1 MOI	IFN-β (mean-SD)	Comparison of serotypes	Accepted
5	In vitro susceptibility to rhinovirus infection is greater for bronchial than for nasal airway epithelial cells in human subjects	2009	Atopic asthmatics (6) - NIH; Non-atopic healthy (5); Demo provided; Non-smokers; w/o ICS	RV-16; 1 MOI	IL-1a, IL-6, RANTES, IP-10 (median-IQR); 48 hrs	Differentiated at ALI	Accepted

6	Rhinovirus-induced modulation of gene expression in bronchial epithelial cells from subjects with asthma	2010	Atopic asthmatics (9); Non-atopic healthy (9); Demo provided; Maybe lesser	RV-1A; 10 MOI	IL-1b, IL-6, IL-8 (mean-SD)	Mainly genes	Accepted
7	Exogenous IFN-β has antiviral and anti-inflammatory properties in primary bronchial epithelial cells from asthmatic subjects exposed to rhinovirus	2011	Atopic asthmatics (8); Healthy (6); Demo provided; w, w/o ICS	RV-1B	IL-6, IL-8, RANTES, IP-10 (box plot w dots); 24, 48, 72 hrs	Healthy controls no values; Different cytokine different sample size	Rejected: Controls are UV irradiated samples
8	Deficient antiviral immune responses in childhood: Distinct roles of atopy and asthma	2012	Atopic asthmatics (8); Non-atopic healthy (9); Demo provided	RV-16	IL-8, IFN-β, IFN-λ (box plots)	Asthma and Healthy (Subgrouped atopic and non-atopic); Not all detected IFN	Accepted
9	Transforming growth factor-beta promotes rhinovirus replication in bronchial epithelial cells by suppressing the innate immune response.	2012	Asthmatics (15); Healthy (23)	RV-1B	TGF-β (box plot)		Rejected: No comparison with other studies

10	Impaired innate interferon induction in severe therapy resistant atopic asthmatic children	2013	Atopic asthmatics (11); Non-atopic healthy (11); Demo provided	RV-16; RV-1B	Il-6, IFN-β, IFN-λ (dot plot)	Severe therapy resistant asthma	Accepted
11	Rhinovirus-induced interferon production is not deficient in well controlled asthma	2013	Atopic asthmatics (10); Non-atopic healthy (12); Demo provided,; w w/o ICS	RV-1B; 1 MOI	IFN-β, IFN-λ (dot plot)	RV-16 only mRNA	Accepted
12	TLR3 and MDA5 signalling, although not expression, is impaired in asthmatic epithelial cells in response to rhinovirus infection	2013	Atopic asthmatics (12) - GINA ; Non-atopic healthy (8); Demo provided; Non-smokers; w ICS	RV-1B; 20 MOI	IL-6, IL-8, IP-10, IFN-λ (mean-SEM)		Accepted
13	Budesonide and formoterol effects on rhinovirus replication and epithelial cell cytokine responses	2013	Atopic asthmatics (9); Non-atopic healthy (8); Demo provided; w ICS	RV-16 (5 x 10^6 PFU/mL)	TNF-α, IL-8, IP-10 (mean-CI); geometric mean and arithmetic mean	IFN-β & IFN-λ not detected with ELISA	Rejected: No comparison among cytokines
14	Rhinovirus induced IL-25 in asthma exacerbation drives type-2 immunity and allergic pulmonary inflammation	2014	Atopic asthmatics (10) - moderate; Non-atopic healthy (10); Demo provided	RV-1B; 2 MOI	IL-25 (dot plot)	Another human experimental study	Rejected No comparison with other studies

15	Phenotypic Responses of Differentiated Asthmatic Human Airway Epithelial Cultures to Rhinovirus	2015	Asthmatics (6); Healthy (6); Demo provided; Smokers and non-smokers	RV-16 (5 x 10^5 PFU / 50uL)	IL-1A, IL-1RA, IL-6, IL-7, IL-8, IL-12p40, IP-10, RANTES, fractalkine, MCP-1, MCP-3, MDC, TNF-a, GCSF, PDGFBB, INF-a2; log2 (mean-SD)	Atopic status not determined; Gene profile	Accepted
16	Impaired airway epithelial cell responses from children with asthma to rhinoviral infection	2016	Atopic asthmatics (25) - mild; Non-atopic healthy (34); Demo provided; w ICS	RV-1B (5 x 10^^5 TCID/mL)	IL-1b, IL-6, IL-8, IP-10, RANTES, IFN-β, IFN-λ (mean-SD)		Rejected: Number of subjects not clearly stated
17	Innate Immune Response to Viral Infections in Primary Bronchial Epithelial Cells is Modified by the Atopic Status of Asthmatic Patients	2018	Asthmatics (10) - atopic and non-atopic; Non-atopic healthy (9); Demo provided; w ICS	RV-1B; 0.1 MOI	IP-10, IFN-λ1 (median-IQR)	Atopic and non-atopic same group	Accepted

Ex vivo PBMC studies							
No.	**Title**	**Year**	**Comparison (Sample size)**	**RV**	**Cytokine/Chemokine**	**Remark**	**Acceptance**
1	A defective type 1 response to rhinovirus in atopic asthma	2002	Atopic asthmatics (7) - mild-to-moderate - physician + skin prick test; Non-atopic healthy (7); Age: 20-57yrs; 3men-4 women; 2 taking ICS; No reported colds	RV-16; 0.01, 0.1, 1, 10 MOI (1 MOI for time points)	IL-4, IL-10, IL-12, IFN-γ; 6, 24, 48 hrs; Mean (SE)		Rejected: No comparison with cytokines from other papers
2	Modulation of the epithelial inflammatory response to	2007	Atopic asthmatics (12) - 2004 GINA; Non-atopic healthy (12);	RV-16 or RV-1B; 5 MOI	RANTES, IL-6, IL-8, IL-10, IFN-γ, TGF-β1;	Only in Scopus	Rejected:

	rhinovirus in an atopic environment		Age: 19-40yrs; 8 men-4 women; Non-smokers; No ICS; No reported colds		48 hrs; log Median (IQR); Before and after		Cell culture study by conditioning with media obtained from RV infected PBMCs
3	Budesonide and Formoterol Reduce Early Innate Anti-Viral Immune Responses In Vitro	2011	Atopic asthmatics (12); Non-atopic healthy (12); Demo provided;	RV-16; 1 MOI	IL-6, IP-10, IFN-α; Median (IQR)		Accepted
4	Peripheral Blood Mononuclear Cells from Patients with Bronchial Asthma Show Impaired Innate Immune Responses to Rhinovirus in vitro	2012	Asthmatics (39); Healthy (7); Demo provided	RV-14; 1 MOI	IFN-α, IFN-γ, IL-6, IL-10, TNF-α, sFasL; Mean (SEM)	Healthy adults with history of childhood asthma; Different age groups; Not all atopic	Rejected: Multiple age groups, comparison is complex
5	Rhinovirus 16–induced IFN-α and IFN-β are deficient in bronchoalveolar lavage cells in asthmatic patients	2012	Atopic asthmatics (22); Non-atopic healthy (20); Demo provided; w and w/o ICS	RV-16, RV-1B; 1 MOI	IFN-α, IFN-β	Another *ex vivo* BAL cellls	Accepted
6	Asthma is associated with multiple alterations in anti-viral innate signalling pathways	2014	Atopic asthmatics (22); Non-atopic healthy (20); Demo provided; w and w/o ICS	RV-16; 5 MOI	IP-10, IFN-α; log Median (IQR)	IQR in the form of error bar	Accepted
7	Rhinovirus induction of fractalkine (CX3CL1) in airway and peripheral blood mononuclear cells in asthma	2017	Atopic asthmatics (15) - Mild; Non-atopic healthy (15)	RV-16, RV-1B; 1 MOI	Fractalkine; Mean (SEM)	Another *ex vivo* BAL cells; Human Experimental	Rejected: No comparison with other studies
8	Interleukin 33 Selectively Augments Rhinovirus-Induced Type 2 Immune Responses in	2018	Asthmatics (14); Healthy (22); Non-smokers; w w/o ICS	RV-16; 1 MOI	IL-5, IL-13, IFN-γ; Mean (SEM)	Atopic and non-atopic in both asthmatics and healthy	Rejected: No comparison with other studies

	Asthmatic but not Healthy People						
9	Similar colds in subjects with allergic asthma and nonatopic subjects after inoculation with rhinovirus-16	2009	Atopic asthmatics (20); Non-atopic healthy (17); Demo provided	RV-16	IFN-α, IFN-γ, IL-6, IL-10, Median (IQR)	Refer to human experimental study no. 5	Rejected: Protein level not provided

Human Experimental studies							
No.	**Title**	**Year**	**Comparison (Sample size)**	**RV**	**Cytokine/Chemokine**	**Remark**	**Acceptance**
1	Rhinovirus induced IL-25 in asthma exacerbation drives type-2 immunity and allergic pulmonary inflammation	2014	Atopic asthmatics (28); Non-atopic healthy (11); Demo provided	RV-16; 100 TCID50	[Nasal Fluid] IL-25 (dot plot)	Refer *ex vivo* PBEC study no. 14	Rejected: OVA treated mice, no comparison study
2	Rhinovirus induction of fractalkine (CX3CL1) in airway and peripheral blood mononuclear cells in asthma	2017	Atopic asthmatics (25) - 11 mild, 14 moderates; Non-atopic healthy (10)	RV-16	[BAL Fluid] Fractalkine (dot plot); Day-14 and Day 4	Refer *ex vivo* PBMC study no. 7	Rejected: No compared cytokine
3	Rhinovirus-16 Colds in Healthy and in Asthmatic Subjects	1998	Atopic asthmatics (11); Non-atopic healthy (10)	RV-16	[Nasal Fluid & Sputum] IL-6 and IL-8 (mean-SD)	Some cytokines are not detected in all patients	Accepted
4	Interleukin-1beta and interleukin-1ra levels in nasal lavages during experimental rhinovirus infection in asthmatic and non-asthmatic subjects.	2003	Atopic asthmatics (25); Non-atopic healthy (12);	RV-16	[Nasal Fluid] IL-1ra, IL-1b, IL-8 (dot plot)		Rejected: Nasal measurements only
5	Similar colds in subjects with allergic asthma and nonatopic	2009	Atopic asthmatics (20); Non-atopic healthy (17); Demo provided	RV-16	[Nasal Fluid] IL-6, IL-8, MCP-1, RANTES	Another *ex vivo* PBMC	Rejected:

	subjects after inoculation with rhinovirus-16						Cytokines not compared between asthmatics and healthy controls
6	The Role of IL-15 Deficiency in the Pathogenesis of Virus-Induced Asthma Exacerbations	2011	Atopic asthmatics (10); Non-atopic healthy (15); Demo provided	RV-16	[BAL Fluid] IL-15 (dot plot)	Another *ex vivo* BAL cells	Accepted
7	CXC chemokines and antimicrobial peptides in rhinovirus-induced experimental asthma exacerbations	2014	Atopic asthmatics (10); Non-atopic healthy (15); Demo provided	RV-16	[BAL Fluid] CXCL1/GRO-α, CXCL2/GRO-β, CXCL5/ENA-78, CXCL6/GCP-2, CXCL7/NAP-2, and CXCL8/IL-8 (dot plot) mean-SD and median-IQR	1 out 10 asthmatics not atopic	Accepted
8	IL-33–Dependent Type 2 Inflammation during Rhinovirus-induced Asthma Exacerbations In Vivo	2014	Atopic asthmatics (28); Non-atopic healthy (11); Demo provided	RV-16	[Nasal Fluid] IL-4, IL-5, IL-13, IL-33 (dot plot)		Rejected: No healthy comparison for bronchial samples
9	A Comprehensive Evaluation of Nasal and Bronchial Cytokines and Chemokines Following Experimental Rhinovirus Infection in Allergic Asthma: Increased Interferons (IFN-γ and IFN-λ) and Type 2 Inflammation (IL-5 and IL-13)	2017	Atopic asthmatics (28); Non-atopic healthy (11); Demo provided	RV-16	[Nasal Fluid & Bronchial Fluid] IL-2, IL-4, IL-5, IL-13, IL-15, IP-10, CXCL-11/ITAC, CCL-17/TARC, eotaxin, eotaxin-3, IFN-γ, IFN-λ (dot plot)	Different sample size for nasal and bronchial fluid	Accepted