Immunological and Inflammatory Biomarkers of susceptibility and severity in Adult RSV Infections

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Abstract:

Background: Respiratory syncytial virus (RSV) is a single stranded negative-sense RNA virus of the *orthopneumovirus* family, best known as the most common cause of bronchiolitis in young infants. 50% of children are infected in their 1st year of life, with hospitalization rates between 2-10%. However, there is growing evidence that RSV is also a significant pathogen in older adults, particularly in those with comorbidities. Validated biomarkers anticipating or reflecting severity of RSV disease would benefit diagnostics, treatment decision making and the targeting of prophylactic interventions. This review summarizes existing knowledge of biomarkers for RSV disease in adults.

Methods: A literature review was performed using the search databases Ovid Medline, Embase, Global health, Scopus and Web of Science for articles published between 1946 and October 2016. Nine articles were highlighted from this search and nine more were added from other sources.

Results: From observational studies of natural infection and challenge studies in volunteers, biomarkers of RSV susceptibility or disease severity in adults were: 1. lower anti-RSV neutralizing antibodies, where neutralizing antibody (and local IgA) may be a correlate of susceptibility/severity; 2. RSV-specific $CD8^+$ T cells in bronchoalveolar lavage (BAL) fluid pre-infection (subjects with higher levels had less severe illness); 3. Elevated Interleukin(IL)-6, IL-8 and Myeloperoxidase (MPO) levels in the airway are indicative of severe infection, including in patients with chronic obstructive pulmonary disease (COPD).

Conclusions: While RSV disease has been well studied in children, factors that determine susceptibility to and severity of RSV disease in adults have not been well defined. However, respiratory mucosal antibodies and CD8+ T cells appear to contribute to preventing infection and modulation of disease severity. Further studies of RSV pathogenesis in at-risk populations, such as the frail elderly and those with respiratory comorbidities are needed.

Key Words: RSV, Biomarker, Adult, Respiratory Syncytial Virus, Severity, RESCEU, COPD, MPO, IL-6, neutralizing antibody

Introduction

Although known primarily as a pediatric virus, the burden of RSV disease amongst adults is increasingly recognized [1]. Some studies have shown that the annual prevalence of a symptomatic infection is up to 10% in high risk adults (including adults >65 years old and those with significant comorbidities) [2]. It is estimated that approximately 14,000-17,000 elderly people die and 170,000 are hospitalized annually in the USA as a result of RSV infection [3, 4]. Additionally, a modeling study performed on data from the UK estimates that RSV accounts for over 17,000 hospitalizations and 8,000 deaths in people over 18 years old. Of those deaths, 93% were in those over 65 years old [5]. There has been substantial progress in the development of new vaccines and immunoprophylactic therapies against RSV in recent years [4]. However, some of the vaccine candidates may only be suitable for maternal or pediatric administration, and none are very close to licensure [4]**.** This review was conducted to summarize the current literature regarding biomarkers that correlate to RSV disease susceptibility and / or severity.

RSV is encountered throughout life. The first infection in infancy can result in severe bronchiolitis requiring ICU treatment [1], however re-infection of a healthy adult might result in only minor symptoms [6]. For this reason, it is important to look at biomarkers for RSV separately in these two groups. The mechanisms that underlie RSV susceptibility and pathogenesis in adults along with the influence of age and co-morbidities, are incompletely understood. Particularly, innate and adaptive anti-pathogen immune responses wane in the elderly, along with chronic low-level immune activation known as “inflammaging” [**7**], a process that may contribute to RSV susceptibility and severity in later life [6]. Studies have investigated the role of innate immunity, antibodies and cellular immunity in the host

response to RSV infection. Such factors may protect against infection or disease severity, though an overexuberant immune response could contribute to disease. Viral replication in the airway has similarly been investigated as a contributor to disease severity. In this article we review studies of these immunological and viral components in natural and experimental RSV infections of adults.

Methods

Initially we searched using a combination of terms ('Human respiratory syncytial virus', 'respiratory syncytial virus' or 'RSV') in Ovid Medline, Embase, Global health, Scopus and Web of Science databases for articles published between 1946 and October 2016. Of the 10,820 abstracts reviewed, 27 papers described studies in adults of which 9 articles contained information on biomarkers of susceptibility or severity of RSV infection in adults. An additional 9 articles known to the authors, published after October 2016, or identified from the references of incorporated papers were also added. We selected publications that delineated factors that reflect susceptibility to, or severity of, RSV infection but excluded studies focusing on methods of RSV viral detection, *in vitro* studies, those analyzing only autopsy specimens, or studies focusing only on pediatric patients or animal models (see Figure 1). No restriction on study design or publication were initially applied. The inclusion and exclusion criteria of studies are listed in table 1. Each included study was independently reviewed by two authors using the Cochrane tool for quality assessment [8].

Results

Humoral Immunity

Antibodies are a central component of the immunological repertoire and are associated with protection from infection from numerous respiratory pathogens. Several studies have investigated the association between RSV infection, disease severity and antibody titers. Particular attention has been given to humoral immunity in elderly and comorbid populations, due to the higher prevalence of severe disease in these groups.

Following natural RSV infection serum IgG levels are known to increase. Falsey et al found that 79% of adults >65 years old had a greater than 4-fold increase in RSV-specific serum IgG compared to 64% of adults <50 years old [9], indicating that the humoral immune response of older adults (>65 years) appears intact in response to natural infection. Although older adults (>65 years) can respond to infection, a low baseline antibody titer (the lowest third of pre-infection neutralizing antibody titers) is associated with an increased susceptibility to symptomatic infection. Luchsinger *et al* observed that a lower RSV serum neutralizing antibody titer at hospital admission amongst adults with community acquired pneumonia (CAP) was associated with an increased likelihood of the CAP being RSV associated (RSV/A 8.1 vs 8.9, and RSV/B 9.3 vs 10.4; $P<0.02$ for both) [10]. Other reports also show an inverse correlation between anti-RSV neutralizing antibody levels in the serum and RSV associated hospitalization in the elderly population [11, 12]. Walsh et al indicated a strong association between RSV infection and low serum neutralizing antibody titers (OR 5.89 CI 1.69-20.57 $P=0.006$) in elderly and high-risk adults (those with symptomatic cardiopulmonary disease) [13]. The importance of neutralizing antibody in protection from RSV has been supported by a human infection challenge study which showed that infected healthy young adults had slightly lower serum micro-neutralizing antibody (MNA) prior to challenge than those who remained uninfected (MNA titers: 9.5log2 versus 10.6log2, $P=0.04$).

The same study also found lower serum RSV F-protein IgG titers (15.2log2 versus 16.4log2, *P*=0.02) in those infected than in those who did not become infected with the virus [14]. Notably, in these young adults, not all infected subjects showed a rise in antibody levels in the serum. Serum antibody levels and neutralizing antibody titers increase after RSV infection [15, 16] but this increase is short-lived, with an approximately two-fold decrease in micro-neutralization titers every 6 months [16]. Interestingly, study participants who did not become infected had a significantly slower decline in antibody over time. This rapid antibody decay, following transient increases post-RSV infection may reflect the "immune amnesia" described with RSV, which permits rapid reinfection, even with antigenically similar viruses [6, 17]. In an RSV challenge study by Lee et al, 8 (67%) of the 12 participants who became infected had a ≥4-fold rise in serum anti-F antibodies and 7 (58%) had ≥4-fold rises in anti-G antibodies [14]. RSV challenge studies have also provided supportive evidence for the important role of mucosal antibody in RSV prevention, where nasal IgA levels (but not serum IgG) are particularly associated with protection (OR=1.9 CI 1.2-3.4 *P*<0.05) [15].

In addition to conferring protection from infection, trends have been observed between lower neutralizing antibody titers and the severity of an RSV infection (*P*=0.07) [18]. By contrast, RSV challenge studies in young adults indicate that once an infection occurs, antibody levels have little impact on disease severity [15, 19]. However, such challenge studies result in mild disease with limited lower airway symptoms and may incompletely reflect the spectrum of natural disease severity. In addition to demonstrating the role of neutralizing antibodies to RSV protection, Walsh et al noted that chronic pulmonary disease was particularly associated with RSV infection (odds ratio 3.97, independent of other factors), indicating that underlying pulmonary health is an important contributor to severity of RSV infections [13]. Nasal IgA has been reported to inversely correlate with peak nasal RSV load during natural infection, where RSV-G specific antibodies were most closely correlated [20]. Similarly,

Cherukuri et al reported that elderly adults with high titers of RSV F-specific nasal IgA were protected from severe RSV illness [11].

Studies have also investigated the association of age with anti-RSV antibody levels. Some studies have demonstrated that nasal RSV F-specific IgA titers were similar between young and older adults [11], as were serum RSV specific IgG levels when measured by immunoassay or MNA [9]. Conversely, other studies have observed decreased MNA titers in older age, despite sustained total RSV antibody levels [9, 21]. A further study investigated viral, humoral and mucosal immunity in protection against natural RSV infection in 1284 prospectively enrolled participants from 4 groups; 1) healthy adults >65 years old, 2) healthy adults 19-40 years old, 3) adults with symptomatic cardiopulmonary conditions and 4) nursing home residents. Symptomatic RSV infections (from mild upper-respiratory illness to pneumonia) were observed in 67 participants, of which 5 were hospitalized [22]. These RSV-infected subjects had significantly lower pre-infection titers of nasal IgA (r=0.58–0.76; *P*=0.0001) and serum IgG against RSV-F, -Ga and -Gb proteins (r=0.54–0.80; *P*=0.0001) and lower serum neutralizing antibody titers. This study could not determine a specific antibody titer predictive of protection from symptomatic RSV infection, although subjects with antibody titers in the highest quartile were approximately 3 times less likely to develop a symptomatic infection [22].

Together, these studies indicate that antibody, particularly IgA in respiratory secretions, provides some protection against RSV infection but may do little to influence disease severity. RSV prefusion (Pre-F) antibodies have recently been implicated as important markers of protection in infants [23] but there is a noted lack of data regarding their role in protection from infection in adults. Neutralization is the main functional test performed in these studies, though other antibody effector functions including antibody-dependent cellular cytotoxicity are under-studied and may contribute to preventing RSV infection or

limiting disease severity. Boosting of antibodies against RSV following infection is short-lived, possibly reflecting "immune amnesia" following infection.

Cellular Immunity

In addition to antibody-mediated protection against RSV infection, the contribution of cellular immunity to protection against infection and in limiting disease severity have also been studied. Although not covered in depth by this review *in vitro* studies have demonstrated that antigen presentation of peptides from most RSV proteins can drive T cell cytotoxicity, with peptides from M2 and SH considered immunodominant [24, 25].

Interferon gamma (IFN-γ) production in response to the RSV F-protein by peripheral blood mononuclear cells (PBMCs) and T cells from elderly donors was significantly lower than young adults (1,250 +/- 420 vs 180 +/- 80 $P<0.001$) [11]. RSV-specific CD8+ T cells were also less abundant in the elderly, relative to younger adults [11, 26, 27]. This decreased frequency and function of RSV-specific T cells in older age may contribute to disease severity, and potentially the rate of viral clearance in the elderly. The central role of cellular immunity was supported by RSV challenge studies, which examined the cytotoxic CD8+ T cell responses in 49 healthy adult volunteers, half of whom had serial bronchoscopies and bronchoalveolar lavage (BAL) for the collection of cells [28]. Interestingly, those patients with greater pre-RSV challenge BAL CD8+ RSV specific T cell levels had less severe infections and a lower viral load ($P=0.0142$ r=-0.691) [28]. CD8+ RSV-specific T cells were less frequent in the blood and no significant correlation was observed between these circulating T cells and RSV severity or viral load, highlighting the importance of collecting samples from the site of disease. There was, however, no significant correlation between CD8+ RSV-specific T cells in baseline BAL and susceptibility to infection, indicating that CD8+ T cells within the airway may not be a crucial contributor to preventing infection but act to

limit disease severity. In addition, this study showed that even in those participants with mild infection scores there was still marked airway inflammation on bronchoscopy, which was maintained after resolution of symptoms in some cases. Interestingly, in the study by de Bree et al, where 31 healthy controls were compared to 9 chronic obstructive pulmonary disease (COPD) patients, it was found that RSV-specific CD8+ T cells were undetectable in the blood of those COPD patients [27].

The relative anergy of PBMCs and T cells from elderly volunteers to RSV proteins/epitopes [11, 29] may be a feature of inflammaging, associated with chronic inflammation [30]. Indeed, RSV-specific T cells have been reported to transition to a Th2 phenotype with age, in addition to increasing Treg frequencies [31]. The combination of these factors may significantly impede the anti-viral immune response to RSV in the elderly, thereby contributing to severity.

Viral Load

Human infection challenge studies have documented infection rates between 50-80% of volunteers exposed, with close temporal and scalar associations between measures of viral load in the upper respiratory tract and symptoms of disease [19, 28, 32-35]. Whilst these studies of healthy young adults provide unique insights into RSV pathophysiology, correlates of protection and predictors of disease severity, the ability to extend the findings of such studies to at risk groups remains questionable. Studies of natural infection in adults have observed that peak nasal viral load was not significantly different between hospitalized and out-patient managed RSV cases [20, 36]. However, the duration of viral shedding was significantly longer in hospitalized cases compared to out-patients (13.1 vs 9.8 days; P=0.003) [20]. Amongst hospitalized patients, those requiring mechanical ventilation for respiratory failure had significantly higher peak viral loads; indeed, high RSV load represented an independent risk-factor for respiratory failure in multivariable analyses [36, 37].

Further studies that perform detailed longitudinal quantification of RSV to define peak viral load, the kinetics of viral clearance and progression of symptoms in at risk adult patients are needed before the findings of RSV challenge studies can be confidently extended to these groups. However, the available data on viral load in naturally infected at-risk patients indicates that viral load is indeed a correlate of disease severity. Conducting challenge studies in at-risk groups has not yet been done. Such studies raise ethical and volunteer safety questions that require close consideration but would offer unique insights to disease pathogenesis in this population.

The Innate Inflammatory Response

In addition to the adaptive humoral and cellular immune responses, innate immune responses may protect against infection and contribute to RSV clearance; conversely, an over-exuberant response may contribute to disease pathogenesis [6]. Comparing adults (≥21 years old) hospitalized with RSV infection to out-patient managed cases, nasal levels of IL-6 (OR 2.2 CI 1.2-4.2 *P*=0.01) and MIP-1α (OR 9.1 CI 0.95–87.6 *P*=0.06) were higher in the hospitalized cohort, where increased IL-6 levels remained associated with severe disease after multivariable analysis [20]. Sputum samples from stable COPD patients, taken over a 2-year period, demonstrated that 32.8% of patients tested positive for RSV at some point during the study [38]. Interestingly, those patients from whom >50% of samples were RSV-positive (18 patients) had a faster decline in respiratory function (measured by forced expiratory volume in 1 second - FEV1) during the study period, and elevated levels of inflammatory cytokines (IL-6, IL-8 and MPO) in the airway [38]. This study raises the possibility of chronic RSV infection (or greatly diminished viral clearance) in this at-risk COPD population, as previously suggested [39] [40].

Discussion

This review highlights the importance of local mucosal immunity in protection against RSV disease. No

228 study was identified that investigated BAL specimens from older adults or comorbid groups, resulting in
229 a significant gap in our knowledge of lower airway immunity to RSV in these populations [41]. However,
230 the probable reason for such studies not yet having been undertaken is that routine lower airway
231 sampling is clinically challenging, and bronchoscopy can be harmful to at-risk adults. Community cohort
232 studies have been undertaken that do include frail elderly and at-risk populations. Their characteristics
233 have been tabulated (table 3).
234
235 There is a relative paucity of data on biomarkers which indicate severity, duration of illness, or
236 susceptibility to symptomatic RSV infection in adults. However, existing data highlight some protective
237 pathways for further analysis, particularly sustained high titer anti-RSV neutralizing antibodies, RSV-
238 specific IgA and CD8+ T cells in the respiratory tract. Associations have been noted between high viral
239 load and disease severity in natural infection and RSV challenge studies, indicating that viral load may be
240 a correlate of disease severity. However, this association is incomplete and is influenced to some degree
241 by pre-existing immunity and underlying health. Age and comorbidities (particularly respiratory and
242 cardiovascular) have been documented to influence the severity of an RSV infection [42], where a
243 possible decline in cellular immunity with age may permit higher infection rates and/or severity in the
244 elderly (Table 2). Elevated levels of inflammatory mediators (including IL-6, IL-8 and MPO) in the airway
245 of chronic/extended or recurrent RSV infection are associated with faster lung function decline in COPD
246 patients. If chronic airway inflammation is present at baseline (e.g. in COPD patients), then the immune
247 and inflammatory response to RSV may be particularly debilitating but unpredictable.
248
249 There are limitations with this review. The initial search did not identify some articles known to the
250 authors and was conducted in October 2016, with literature on the subject constantly being updated.
251 We have attempted to include the relevant studies that this search missed and those that were more

recently published, but some may have been omitted.

In conclusion, additional studies are needed to improve our understanding of RSV pathogenesis in specific at-risk populations, including the frail elderly and those with respiratory comorbidities. It would be of particular interest to determine the kinetics of viral clearance and the anti-RSV immune response in the upper and lower airways of such populations. Examples of some specific research questions have been tabulated (Table 4). Filling such fundamental knowledge gaps appears crucial to understanding RSV pathogenesis and the development of successful anti-RSV therapies in vulnerable adult populations.

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Definitions

RSV infection was identified definitively on a diagnostic test of a body fluid including: PCR; viral culture or antigen test. Severity of RSV infection was considered appropriate when authors stated their definition or used at least one of the following terms: lower-respiratory tract infection; admission or hospitalisation due to RSV infection; moderate or severe bronchiolitis; pneumonia or the need for respiratory support (non-invasive or mechanical ventilation). Biomarkers were defined as any traceable biological parameter/substance that was measurable. Further classification into different groups was performed in order to regroup the broad diversity of markers identified in the literature.

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Footnotes

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418 Table 1: Eligibility and exclusion criteria

Inclusion	**Exclusion**
● Human respiratory syncytial virus studies in adults ● Severity of RSV infection assessed ● Biological marker investigated ● Studies written in English, French, Spanish, Italian or Portuguese	● Studies in animal models ● *In vitro* studies ● Studies exclusively in children ● Studies of treatment, diagnostics or epidemiology of RSV infection ● Absent definition of disease severity ● Studies without a definitive RSV diagnosis ● Studies focusing on viral characteristics. ● Literature reviews

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423 Table 2: Summary of RSV Biomarkers in Adults

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Marker of Infection Severity		**Marker of Susceptibility for Infection**	
Systemic Markers	**Airway Markers**	**Systemic Markers**	**Airway Markers**
Low RSV neutralizing antibody titer in serum [18] (Trend P=0.07)	Low levels of RSV CD8 + T cells in BAL [28] (P=0.0142 r= -0.691)	Low RSV neutralizing antibody titer in serum **[**10] (P≤ .028) [12] (P = 0.008 and P = 0.01 for RSV/A and RSV/B respectivley [13] (P=0.018) [14] (*P* = 0.04)	Low IgA to RSV F, Ga + Gb proteins in nasal secretions [22] (*r* = .58–.76; *P* = .0001)
	High levels of IL-6 in airway [38] (P < 0.001)	Low IgG to F, Ga + Gb RSV protein levels in serum [22] (*r* = .54–.80; *P* = .0001) [14] (only F-Protein *P* = 0.02)	Low IgA to RSV in nasal mucosa [15] (*P* < 0.05) [19] (P=0.292)
	High levels of IL-8 in airway [38] (P= < 0.001)		Low IgA to RSV F-Protein in nasal mucosa [15] (*P* < 0.05)

	High levels of MPO in airway [38] (P= < 0.001)		
	High Viral Load [28] (P=0.003) [33] (P=<0.05) [34] [35] (P=0.0340) [37] (*P* =0.011)		
	Viral shedding in nasal secretions for longer [20] (13.1 vs 9.8 days; *P* = 0.003)		
	Low levels of IgA to RSV Ga + Gb in nasal mucosa [20] (Ga P=0.003 Gb P <0.0001) [34] (P=0.03)		
	Higher levels of nasal IL-6 [20] (OR 2.2 (1.2-4.2) P=0.01) and MIP-1α [20] (OR 9.1 (0.95 – 87.6) P= 0.06)		

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430 Table 3: Study Characteristics

Author, year	**Prospective Study**	**Natural Infection**	**Healthy Controls / Younger Adults Included**	**High Risk / Co-Morbid Adults Included**	**Older Adults Included**
Falsey AR, 1999 [9]	Yes	Yes	Yes	Yes (nursing home eligible but living in the community)	Yes
Cherukuri A, 2013 [11]	Yes	No	Yes	No	Yes
Falsey AR, 1998 [12]	Yes	Yes	Yes	Yes (nursing home eligible but living in the community)	Yes
Walsh EE, 2004 [13]	Yes	Yes	Yes	Yes (Underlying symptomatic	Yes

				cardiopulmonary conditions)	
Lee FE-H, 2004 [14]	Yes	No	Yes	No	No
Habibi MS, 2015 [15]	Yes	No	No	No	No
Falsey AR, 1992 [18]	Yes	Yes	No	Yes (Nursing Home Residents)	Yes
Bagga B, 2015 [19]	Yes	No	Yes	No	No
Walsh EE, 2013 [20]	Yes	Yes	Yes	Yes	Yes
Walsh EE, 2004 [22]	Yes	Yes	Yes	Yes (CHF / COPD)	Yes
Jozwik A, 2015 [28]	Yes	No	Yes	No	No
Duncan CB, 2009 [36]	Yes	Yes	Yes	Yes	Yes
Lee N, 2015 [37]	Yes	Yes	Yes	Yes	Yes
Wilkinson TM a, 2006 [38]	Yes	Yes	No	Yes (all patients had COPD)	Yes

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434 Table 4– Future Research Options

Limitations with current research	**Possible future research options**
Challenge Studies have previously been only in young healthy adults	Challenge studies in older adults and co-morbid populations
Mainly upper airway studies have been performed in the observational groups	Lower airway sampling in infected adults is needed with comparison between upper and lower airway biomarkers
Virus has been found at multiple timepoints in the same patient. It is not known if this is RSV recurrence or 'chronic infection'	Genotyping of RSV found in the same patient at multiple timepoints may confirm if infection with a new strain is occurring or if the virus is not being cleared
Viral clearance is poorly understood	Investigate which host or virologic factors influence the rate of viral clearance and disease severity, e.g. the kinetics of viral clearance and anti-viral immune responses.
	Investigate the role of pre-f antibodies in disease susceptibility in adults
	Why is infection severe in some populations and mild in others and are the kinetics of viral clearance distinct between these groups

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