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# Nasal Immune Responses to Human Metapneumovirus

## İnsan Metapnömovirus'una Nazal Bağışıklık Yanıtları

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## Abstract

**Introduction:** Human metapneumovirus (hMPV) with tropism for the respiratory tract epithelium, causes 5–10% of all acute respiratory tract infections (ARI). Patients may be asymptomatic, or exhibit mild upper respiratory tract symptoms or severe bronchiolitis and pneumonia. Determinative diagnostic markers for infection are not well elucidated. This study on nasal immune responses to hMPV aims to understand the mucosal immune responses to hMPV and its implication in disease resolution and pathology.

**Materials and Methods:** A Hospital cross-sectional study design with 196 participants was adopted. Two nasal swabs for viral screening and quantification by RT-PCR and the second for quantification of the cytokines were obtained. Analysis was done using STATA version 13. The test for association between categorical variables was done using Pearson's Chi Square test while the test for association between categorical and continuous variables was done using ANOVA test.

**Results:** hMPV prevalence was 4% with mean hMPV cycle threshold value of 28.34. The entire sample of participants positive for hMPV had median cytokine levels: IL-2, 57.11 (IQR: 34.49–70–49), IL-4, 30.49 (IQR: 8.07–60.84), IL-8, 396.61 (IQR: 81.91–530.71), IL-10, 26.98 (IQR: 7.55–42.65), and IL-12-p70, 44.93 (IQR: 23.59–70.38). There was a positive association between cytokines; IL-10 and IL-12p70 p=0.0005, IL-2 and IL-10 p=0.007, IL-2 and IL-12p70 p=0.015

**Conclusions:** hMPV is not an important ARI etiological factor in infants, its infection induces cytokines with a Th2 bias.

**Keywords:** Human Metaapneumovirus (hMPV), ARI, nasal immune responses, interleukins, determinative, diagnostic

## Öz

**Giriş:** Solunum epitelinde tropizme neden olan Human metapneumovirus (hMPV), tüm akut solunum yolu enfeksiyonlarının (ARI) %5–10'una neden olur. Hastalar asemptomatik olabilir ve bazı hastalarda hafif üst solunum yolu semptomlarına, ciddi bronşiyolit veya zatürreye neden olabilir. Bununla birlikte, enfeksiyon için tanısal belirteçler hala tam olarak açıklanamamıştır. Bu çalışma, HMPV'nin nazal immün yanıtlar, mukozal immün yanıtlar, patoloji ve hastalıkların iyileşmesi üzerindeki etkisini göstermeyi amaçlamaktadır.

**Gereç ve Yöntemler:** Çalışma için 196 kişiden nasal sürüntü örnekleri alındı. Hastaların her birinden nazofarengeal sürüntü çubuğu kullanılarak viral tarama yapılması ve RT-PCR ile miktar tayini için bir ve sitokin tayini için de bir tane olmak üzere toplamda iki örnek alındı. İstatistiksel analizler için STATA 13 yazılımı kullanıldı. Kategorik değişkenler arasındaki ilişkilendirme testi Pearson'ın Chi Square testi kullanılarak, kategorik ve sürekli değişkenler arasındaki ilişkilendirme testi ANOVA testi ile yapıldı.

**Bulgular:** hMPV prevalansı 28,34 ortalama hMPV BT ile %4 idi. HMPV için pozitif olan hastaların tümünün ortalama sitokin düzeyleri vardı: IL-2, 57,11 (IQR: 34,49–70–49), IL-4, 30,49 (IQR: 8,07–60,84), IL-8, 396,61 (IQR: 81,91)-530,71), IL-10, 26,98 (IQR: 7,55–42,65) ve IL-12-p70, 44,93 (IQR: 23,59–70,38). Sitokinler arasında pozitif bir ilişki gözlendi; IL-10 ve IL-12p70 p=0,0005, IL-2 ve IL-10 p=0,007, IL-2 ve IL-12p70 p=0,015

**Sonuç:** HMPV, bebeklerde önemli bir akut solunum yolu enfeksiyonuna yol açan etiyolojik faktör değildir. Bu çalışmada, HMPV'nin en fazla Th2 hücreleri uyardığı ve Th2 sitokinlerinin salınmasını gösterilmiştir.

**Anahtar Kelimeler:** hMPV, ARI, nazal immün yanıt, interlökinler, belirleyici, tanısal

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## Introduction

Acute respiratory tract infections (ARI) are important causes of morbidity and mortality in children globally. The World Health Organization (WHO) estimates acute respiratory infections (ARI) cause nearly one million deaths annually, signifying a fifth of all deaths. In resource scarce countries higher have been recorded, with pneumonia accounting for an estimated 11% of all deaths among children aged below 5 years[1] viral aetiology has

been associated with a substantial proportion of ARI. Viral infections can exacerbate chronic or recurring respiratory conditions, such as asthma, thus posing an additional burden.[2] The role of human metapneumovirus (hMPV) is well established. The viral aetiology of ARI, is associated with a self-limiting upper respiratory tract infection, mild pneumonia or severe pneumonia.[3]

Human metapneumovirus, discovered almost two decades ago by Van den Hoogen in 2001, is an enveloped, negative-stranded RNA virus of approximately 13KB and a member of the *pneumovirinae subfamily* of the paramyxoviruses.[4] Studies suggest that hMPV is responsible for 5–10% of ARIs in neonates and children.[5,6] Based on literature from Kenya, the prevalence of hMPV is placed at 5.7% in refugee populations[7,8] reports a prevalence of 5–15% in selected urban and rural populations.

hMPV infections have been reported mainly vulnerable groups such as children, the elderly and immunocompromised adults[9,10] HMPV is tropic for the respiratory tract epithelium, with infected individuals exhibiting no symptoms, or symptoms ranging from mild upper respiratory tract symptoms to severe bronchiolitis and pneumonia in the lower respiratory tract.[9,11] During hMPV infection; mild upper respiratory illness occurring as Influenza-like Illness (ILI) or severe disease described as Severe Acute respiratory illness (SARI).

Pulmonary inflammation is critical in viral control in the airways. Studies indicate that inflammatory cells such as neutrophils and natural killer cells that have been implicated in infection resolution and immunopathology in active infection.[12] T cell profiles; $CD4^+$ T cells and $CD8^+$ T cells, have been reported to play an antiviral role with the two subsets acting synergistically in hMPV clearance from the lung.[13,14] These T lymphocytes serve to clear active infection, protect against future infection and they are also reported to contribute to clinical disease and lung pathology, although the pathophysiology phenomenon is yet to be fully elucidated[14], yet the potential role of $CD4^+$ (IL-4 producing) and not $CD8^+$ T cells in disease aggravation has been suggested by Darnoit et al.[15]

Phagocytic cells such as neutrophils are recruited into the airways; however, an exacerbated inflammatory response may result in severe lung disease, due to immunopathology.[16] A Finish study demonstrated increased levels of IL-8. IL-8 is a neutrophil chemoattractant[17] in mucus secretions of children infected by the virus. It is therefore important to investigate if this phenomenon will be replicated in the Kenyan population.

Studies have not fully explored the role innate and adaptive immune responses to hMPV infection. Thus, it is important to determine the nasal immune response and inflammatory makers that may correlate with disease severity, with the aim of distinguishing the role of inflammation in hMPV infection. Immune profiles correlated with disease severity will identify the role of immune cells in viral clearance as well as in the infection induced immunopathology.

## Materials and Methods

The study was done at the Moi Teaching and Referral Hospital (MTRH) paediatric outpatient and inpatient units. The second site was Huruma Sub-County Hospital in Eldoret. The laboratory assays were carried out in CDC/KEMRI Nairobi and AMPATH-Eldoret labs.

A hospital cross-sectional study design was adopted. Samples were only collected once at the time of the study. This study design was useful in assessing prevalence, and 196 participants were recruited. Background information was obtained by questionnaire. Scoring of each patient was done according to clinical symptoms, age and number of days with symptoms. Children meeting inclusion criteria and whose parents consented were included into the study. The participants who tested positive for the virus, specific nasal cytokine profiles were quantified and described.

Each study participant was sampled for 2 Nasal (NP) swabs, by use of nasal (Nylon tipped flocked) swab manufactured by COPAN Italia. The swab was inserted into the middle meatus of the nostril and turned carefully three times, then withdrawn. The two NP swab were inserted immediately in viral transport medium (2 mL). Storage done at-80$^0$C prior to virus detection by RT-PCR and cytokines quantification.

### PCR

PCR was carried out with the aim of quantifying viral amounts in the swab along with assessment of swab integrity. Viral nucleic acid was extracted from the nasal swab specimen. QIA amp RNA extraction kit was used following the manufacturer's instruction manual. The extracted RNA was processed by PCR to detect presence of the virus. hMPV was confirmed by RT-PCR using primers

targeted to the conserved region of hMPV nucleocapsid protein. The primers and probes were provided by CDC Atlanta and KEMRI CDC Nairobi. Ag-Path _ID One Step RT-PCR KIT manufactured by Life Technologies was used for PCR. The Real Time PCR cycling conditions for hMPV included Reverse Transcription at 45°C for 10 min, followed by denaturation at 95°C for 10 min, Enzyme activation at 95°C for 0.15 minutes (Cycles), Annealing at 55°C for 1 min, extension at 72°C for 1 min and finally, data collection. The same cycling conditions were used for human RNase P gene (RNP) PCR. Cycle threshold (Ct) represented the number of cycles need for amplification to occur.

### Cytokine Quantification

Cytokines instead of specific immune cells will be quantified as cytokines are known immunomodulators. Cytokines were quantified using Enzyme linked Immunosorbent Assay (ELISA). IL-2, IL-4, IL-8 and IL-12p70 were quantified using Biolegend ELISA Kits thus adopting a sandwich ELISA with Pre-coated with a capture antibody. The manufacture's' manual was followed. The absorbance was measured at 450 nm and standard curves prepared. IL-10 was also quantified by sandwich ELISA whereby the plates were coated with capture antibody and incubated overnight then cytokine was quantified following the Manufacturers' manual. Absorbance was measured at 450 nm and a standard curve was prepared.

### Statistical Analysis

Data analysis was done using Stata version 13 special edition. Categorical variables were summarized as frequencies and the corresponding percentages. Continuous variables were summarized as mean and the corresponding standard deviation (SD) if it assumes the Gaussian distribution. If the continuous variable violated the Gaussian assumptions, then it was summarized as median and the corresponding inter-quartile range (IQR). The assumptions for normality were assessed using Shapiro-Wilks test. Age was categorized as <6 months, 6–12, months, 12–18 months, and >18 months. The test for association between categorical variables was done using Pearson's Chi Square test while the test for association between categorical and normally distributed continuous variables was done using one-way analysis of variance (ANOVA). The relationship between the continuous variables was assessed using Spearman rank correlation coefficient since both or one of the variables violated the Gaussian assumptions. We reported the results and the associated p-values, and 95% confidence limits.

## Results

This is a report of data collected from October of 2013 to January of 2014, 196 participants whose data were included for analysis. The median age was 11 (IQR: 6–17) months with a minimum of 1 month and a maximum of 24 months. Sixty-seven children (34%) were aged less than six months. Relatively, the proportions in all the groups were similar. There were more males (n=104;53%) than females (Table 1) (p=0.785). Male represented 104 (53%), of the study population.

**Table 1.** Sex distribution within each age group

| | Sex | | |
|---|---|---|---|
| **Age** | **Female (n=92)** | **Male (n=104)** | **Total (n=196)** |
| <6 Months | 28 (31%) | 37 (36%) | 65 (34%) |
| 6–12 Months | 21 (23%) | 26 (25%) | 47 (24%) |
| 12–18 Months | 25 (27%) | 24 (23%) | 49 (25%) |
| >18 Months | 18 (19%) | 17 (16%) | 35 (18%) |
| Total | 92 (100%) | 104 (100%) | 196 (100%) |

p=0.785

### PCR Findings

There were eight participants positive for hMPV and whose Ct levels were determined. The average hMPV Ct was 28.37 and average age for these patients was 13.38 months. Gender was equally distributed among these participants. The prevalence of hMPV infection was 4%.

All the participants who had hMPV Ct determined had a cough symptom while 25% weight loss and 37.5% of patients were lethargic. Fifty% of the patients presented with diarrheal symptoms. The average duration of the symptoms was 2.9±1.6 days and the average temperature was 38.04±0.12$^0$C.

Samples were assayed for RNase P (human housekeeping gene) and any sample with RNase P-Cycle Threshold (RNP) Ct<40 was rejected. The average RNP Ct was 24.03±2.68 with a minimum of 18.25 and a maximum of 34.08.

### ELISA Findings

hMPV Ct was (p=0.007 and p=0.015 respectively) correlated with the fever in patients (r=0.3696, p=0.184)

coefficient hMPV Ct was slightly associated with the duration of clinical symptoms (r=0.570, p=0.069).

Association between the cytokine levels and clinical symptoms was calculated by Pearson's Chi square and the cytokine levels were as presented in Table 2. There was no statistically significant association.

There was negative correlation between fever, and IL-2, IL-8, IL-10, and IL12-P70 levels (Table 2). There was a positive correlation between fever and IL-4 implying high levels of IL-4 is positively associated with fever.

There was a statistically significant association between IL-10 and IL-12P70 r=0.9, (p=0.005). IL-2 levels were found to be associated with IL-10, and IL-12P70 levels, (p=0.007 and p=0.015, respectively).

## Discussion

This prospective study aimed to determine prevalence of hMPV and assess specific nasal cytokine profiles in children under the age of two. hMPV is not an important etiological factor of acute respiratory illness with all patients in this study exhibiting mild clinical illness.

Our study demonstrated hMPV circulating amongst the Eldoret populations. hMPV was detected, and the prevalence was placed at one in twenty-five patients, slightly lower than reports from other studies which reported prevalence between 5–10% in refugee populations in Kenya where the peak incidence periods were November and December.[7] Another study in Kenya reported the prevalence in different urban and rural populations to be 5–15%.[8] Data from the study supports the assumption that clinical manifestation associated most commonly with hMPV for the out patients are cough and fever which were exhibited in all patients who presented clinically with mild upper respiratory illness. In our study, those were present in all patients with hMPV-positive samples; similar observations were reported by other studies.[18,19] Nevertheless, lower respiratory infection such as pneumonia can be presented clinically, though we did not have any patients with lower respiratory tract infection.

Quantitative Real-Time PCR (qRT-PCR) was used to quantify the amounts of virus in a clinical specimen and Ct for viral load estimation. Semi-quantitative RT-PCR (qRT-PCR) has been reported to have the potential of estimating infectious virus levels that correlate well with the standard curves using limiting dilutions of the purified PCR amplicons.[20,21] To confirm the specimen integrity, each sample was evaluated for the human housekeeping gene *RNase P (*RNP). Younger children had lower Ct values than that of older children, but this was not statistically significant. Generally, the average hMPV Ct value was low, this, being indicative of higher viral loads. The lower Ct values observed was inconsistent with findings from another study[21], this could be due to the varied swabs used; Nylon vs polyester tipped swabs. It is also possible that the lower Ct values reported in our study were due to the point in time during infection. On correlating the viral load and fever, we report no association this being consistent with findings from a previous study.[22] We report a positive association between Ct values and days since onset though the association was not significant.

Our study characterized markers of inflammation and how they related with specific clinical symptoms. The characterized inflammatory markers were elevated except for IL-12p70 whereas low levels were observed when compared to the normal serum levels in healthy children.[23] It is possible that inflammation leads to resolution of infection by direct antiviral activity or by induction of innate and adaptive immunity. IL-4 hyper responsiveness was observed, and this could be indicative of Th2 bias. It is therefore possible that IL-4 could play an important role in hMPV infection pathogenesis as opposed to viral clearing. This finding is consistent with reports from other studies that reported a Th2 biased

**Table 2.** Association of cytokine levels and clinical symptoms

| Symptom | IL-2 (pg/ml) | IL-4 (pg/ml) | IL-8 (pg/ml) | IL-10 (pg/ml) | IL-12p70 (pg/ml) |
|---|---|---|---|---|---|
| | Median (IQR) | Median (IQR) | Median ((IQR) | Median (IQR) | Median (IQR) |
| Cough (8) | 57.11 (34.49–70–49) | 30.49 (8.07–60.84) | 396.61 (81.91–530.70) | 26.98 (7.55–42.65) | 44.93 (23.59–70.38) |
| Weight-Loss (2) | 38.82 (34.49–43.16) | 33.36 (14.49–52.23) | 403.0 (39.99–766.00) | 35.51 (28.37–42.65) | 57.66 (44.93–70.38) |
| Lethargy (3) | 57.11 (34.49–58.83) | 52.23 (8.07–151.74) | 239.42 (81.91–766.00) | 26.98 (5.26–42.65) | 40.41 (23.59–70.38) |
| Vomiting (1) | 57.11 | 151.74 | 81.91 | 5.26 | 23.59 |
| Diarrhea (4) | 38.82 (32.81–172.59) | 22.49 (10.56–41.36) | 404.69 (218.30–589) | 35.51 (17.96–49.45) | 57.66 (26.31–103.52) |

cytokine response.[24,13] High levels of IL-8 in mild disease observed could indicate that IL-8 is important in infection resolution since, IL-8 is a portent neutrophil chemotactic and activating factor. Moreover, regulated recruitment and clearance of neutrophils is characteristic of competent host defence. IL-8 is thought to play an essential role in pathogenesis of severe RSV infections[25], This however, may not be the case in hMPV, since all the participants had high levels of IL-8 despite the mild disease. Low IL-12p70 levels were observed in our study. IL-12 plays an important role in induction of antiviral interferon; it is likely that this cytokine plays a critical role in viral clearance. Similar findings have been reported whereby, hMPV infection is associated with enhanced expression of Th-1 type (IL-12 and IFN-γ) and antiviral (IFN-β) cytokines.[26] The low levels of IL-12 observed could be explained by inhibition of T cell proliferation by hMPV infected dendritic cells.[27] We also sought to understand the role of IL-10 as a regulatory cytokine during active infection. We observed slightly elevated levels of IL-10. This cytokine was associated with a higher IL-12p70 suggesting, an increase in IL-12p70 was associated with increased IL-10. This may imply the regulatory role of IL-10 on IL-12 secretion. Similar findings have been reported regarding IL-10 levels.[28] IL-2 is vital in promoting clonal expansion of T cells. We found moderate elevation of IL-2. A study reported similar findings with low IL-2 production in adults infected with the virus. The low levels were observed from day 1 of infection to day 12.[29] Lower levels of IL-2 could be attributable to hMPV infection of dentritic cells hampering the capacity to activate T cells[30]; also, hMPV is thought to inhibit DCs function in priming naive T cells.[27] Regarding the association between increased IL-2 and IL-10 with IL12P70 found in our study, it can be proposed that, IL-2 might play a role in expansion of T cell lineage.

In our study, fever was associated negatively with IL-2, IL-8, IL-10 and IL-12P70 but a positively with IL-4. IL-10 is an inhibitory cytokine thus, it's role in fever is expected to abate fever. This could implicate high level of IL-4 to being associated with fever but it is more likely that fever occurred as result of the viral infection rather than higher cytokine levels that was supported by the study indicating that fever was associated with virus-positive exacerbation in infants.[31] IL-8 on the other hand was not associated with fever although IL-8 is known to be pyrogenic.[32]

We sought to understand if duration of disease was in any way associated with the quantities of cytokines secreted by the infected children. IL-2 and IL-8 had a negative correlation while IL-4, IL-10 and IL-12p70 had a positive correlation with symptoms. High levels IL-4, IL-10 and IL-12P70 were found to be associated with the duration of illness. Data from previous study indicated that IL-4 was at the highest level on day 5 of infection[29]; It is therefore plausible to speculate that higher IL-4 levels could be associated with higher duration of illness. The average duration of disease in our study was 3 days. We speculate that these two cytokines are likely to peak at the same time. Data from our study indicated a statistical significant correlation between IL-10 and IL-12P70. In a recent study, it was reported that peak secretion of IL-10 might coincide with viral shedding which peaks between days 5 and 10.[29] Our data indicated no association between the viral load and cytokine quantities. We did not find any correlation between cytokine levels and Ct.

## Conclusions

Data from the study demonstrates circulation of hMPV amongst the population in Eldoret albeit it not being an important etiological factor for ARI and qRT-PCR it is an important diagnostic assay for acute respiratory illness. hMPV infection induces secretion of cytokines in nasal epithelial cells. Elevated cytokine levels indicate that the essential role these inflammatory markers more so IL-8, this highlights the importance of neutrophils in viral clearance leading to disease resolution. hMPV infection leads to IL-4 hyper responsiveness.

### Recommendations

Ct values from PCR could play an important role in diagnosis of upper respiratory tract infections in a clinical setting. Further investigations are needed to assess the role of cytokines and functions in severe cases of hMPV infection.

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## References


1. World Health Organization. Recommendations for management of common childhood conditions. Evidence for technical update of pocket book recommendations. Geneva; 2012 http://www.who.int/maternal_child_adolescent/documents/management_childhood_conditions/en
2. Busse WW, Lemanske RF Jr, Gern JE. The Role of Viral Respiratory Infections in Asthma and Asthma Exacerbations. Lancet 2010;376:826–34. [CrossRef]
3. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. Lancet 2011;377:1264–75. [CrossRef]
4. Biacchesi S, Skiadopoulos MH, Yang L, Lamirande EW, Tran KC, Murphy BR, et al. Recombinant human Metapneumovirus lacking the small hydrophobic SH and/or attachment G glycoprotein: deletion of G yields a promising vaccine candidate. J Virol 2004;78:12877. [CrossRef]
5. Agrawal AS, Roy T, Ghosh S, Chawla-Sarkar M. Genetic variability of attachment (G) and Fusion (F) protein genes of human metapneumovirus strains circulating during 2006–2009 in Kolkata, Eastern India. J Virol 2011;8:67. [CrossRef]
6. Esper F, Boucher D, Weibel C, Martinello RA, Kahn JS. Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. Pediatrics 2003;111:1407–10. [CrossRef]
7. Jamal A, Katz MA, Auko E, Njenga MK, Weinberg M, Kapella BK, et al. Epidemiology of respiratory viral infections in two long-term refugee camps in Kenya, 2007–2010. BMC Infect Dis 2012;12:7. [CrossRef]
8. Omballa V, Cosmas L, Ongus J, Kikwai G, Mwiti W, Bigogo G, et al. Epidemiology of Human metapneumovirus in rural and urban populations of Kenya, 2006–2009. Supplementary paper.
9. Boivin G, Mackay I, Sloots TP, Madhi S, Freymuth F, Wolf D, et al. Global genetic diversity of human metapneumovirus fusion gene. Emerg Infect Dis 2004;6:1154–7. [CrossRef]
10. Sumino KC, Agapov E, Pierce RA, Trulock EP, Pfeifer JD, Ritter JH, et al. Detection of severe human metapneumovirus infection by real-time polymerase chain reaction and histopathological assessment. J Infect Dis 2005;192:1052–60. [CrossRef]
11. Cane PA, van den Hoogen BG, Chakrabarti S, Fegan CD, Osterhaus AD. Human metapneumovirus in a haematopoietic stem cell transplant recipient with fatal lower respiratory tract disease. Bone Marrow Transplant 2003;31:309–10. [CrossRef]
12. Huck B, Neumann-Haefelin D, Schmitt-Graef A, Weckmann M, Mattes J, Ehl S, Falcone V. Human metapneumovirus induces more severe disease and stronger innate immune response in BALB/c mice as compared with respiratory syncytial virus. Respir Res 2007;8:6. [CrossRef]
13. Alvarez R, Tripp RA. The immune response to human metapneumovirus is associated with aberrant immunity and impaired virus clearance in BALB/c mice. J Virol 2005;79:5971–8. [CrossRef]
14. Kolli D, Bataki EL, Spetch L, Guerrero-Plata A, Jewell AM, Piedra PA, et al. T lymphocytes contribute to antiviral immunity and pathogenesis in experimental human metapneumovirus infection. J Virol 2008;82:8560–9. [CrossRef]
15. Darniot M, Pitoiset C, Petrella T, Aho S, Pothier P, Manoha C. Age-associated aggravation of clinical disease after primary metapneumovirus infection of BALB/c mice. J Virol 2009;83:3323–32. [CrossRef]
16. Kolli D, Bao X, Liu T, Hong C, Wang T, Garofalo RP, Casola A. Human Metapneumovirus Glycoprotein G Inhibits TLR4-Dependent Signaling in Monocyte-Derived Dendritic Cells. J Immunol 2011;187:47–54. [CrossRef]
17. Jartti T, Van den HB, Garofalo RP, Osterhaus AD, Ruuskanen O. Metapneumovirus and acute wheezing in children. Lancet 2002;360:1393–4. [CrossRef]
18. Mohamed MSE, Reiche J, Jacobsen S, Thabit AG, Badary MS, Brune W, et al. Molecular Analysis of Human Metapneumovirus Detected in Patients with Lower Respiratory Tract Infection in Upper Egypt. Int J Microbiol 2014;2014:290793. [CrossRef]
19. Reiche J, Jacobsen S, Neubauer K, Hafemann S, Nitsche A, Milde J, et al. Human Metapneumovirus: Insights from a Ten-Year Molecular and Epidemiological Analysis in Germany. PLoS ONE 2014;9:e88342. [CrossRef]
20. Martin ET, Kuypers J, Heugel J, Englund JA. Clinical disease and viral load in children infected with respiratory syncytial virus or human metapneumovirus. Diagn Microbiol Infect Dis 2008;62:382–8. [CrossRef]
21. Fuller JA, Njenga MK, Bigogo G, Aura B, Ope MO, Nderitu L, et al. Association of the CT Values of Real- Time PCR of Viral Upper Respiratory Tract Infection with Clinical Severity, Kenya. J Med Virol 2013;85:924–32. [CrossRef]
22. Houben LM, Coenjaerts FEJ, Rossen JWA, Belderbos ME, Hofland RW, Kimpen JLL, Bont L. Disease Severity and Viral Load Are Correlated in Infants With Primary Respiratory Syncytial Virus Infection in the Community. J Med Virol 2010;82:1266–71. [CrossRef]
23. Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G. Cytokine Levels in the Serum of Healthy Subjects. Mediators Inflamm 2013;2013:434010. [CrossRef]
24. Hamelin ME, Boivin G. Development and validation of an enzyme-linked immunosorbent assay for human metapneumovirus serology based on a recombinant viral protein. Clin Diagn Lab Immunol 2005;12:249–53. [CrossRef]
25. Hall CB. Respiratory syncytial virus and parainfluenza virus. N Engl J Med 2001;344:1917–28. [CrossRef]
26. Herd KA, Nelson M, Mahalingam S, Tindle RW. Pulmonary infection of mice with human metapneumovirus induces local cytotoxic T-cell and immunoregulatory cytokine responses similar to those seen with human respiratory syncytial virus. J Gen Virol 2010;91:1302–10. [CrossRef]
27. Cespedes FP, Gonzalez PA, Kalergis AM. Human metapneumovirus keeps dendritic cells from priming antigen-specific naive T cells. Immunology 2013;139:366–76. [CrossRef]
28. Laham FR, Israele V, Casellas JM, Garcia AM, Lac Prugent CM, Hoffman SJ, et al. Differential production of inflammatory cytokines in primary infection with human metapneumovirus and with other common respiratory viruses. J Infect Dis 2004;189:2047–56. [CrossRef]
29. Talaat K, Karron RA, Thumar B, McMahon BA, Schmidt AC, Collins PL, Buchholz UJ. Experimental Infection of Adults with

Recombinant Wild-Type Human Metapneumovirus. J Infect Dis 2013;208:1669–78. [CrossRef]

**30.** Le Nouen C, Hillyer P, Munir S, Winter CC, McCarty T, Bukreyev A, et al. Effects of Human Respiratory Syncytial Virus, Metapneumovirus, Parainfluenza Virus 3 and Influenza Virus on CD4+ T Cell Activation by Dendritic Cells. PLoS One 2010;5:e15017. [CrossRef]

**31.** Nitin K, Mackay IM, Sloots TP, Masters IB, Chang AB. Respiratory viruses in exacerbations of non-cystic fibrosis bronchiectasis in children. Arch Dis Child 2013;99:749–53. [CrossRef]

**32.** Zampronio AR, Souza GE, Silva CA, Cunha FQ, Ferreira SH. Interleukin-8 induces fever by a prostaglandin-independent mechanism. Am J Physiol 1994;226:R1670–4. [CrossRef]