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TITLE:
Clinical Disease Severity of Respiratory Viral Co-Infection versus Single Viral Infection: A Systematic Review and Meta-Analysis

ABSTRACT:
BACKGROUND: Results from cohort studies evaluating the severity of respiratory viral co-infections are conflicting. We conducted a systematic review and meta-analysis to assess the clinical severity of viral co-infections as compared to single viral respiratory infections. METHODS: We searched electronic databases and other sources for studies published up to January 28, 2013. We included observational studies on inpatients with respiratory illnesses comparing the clinical severity of viral co-infections to single viral infections as detected by molecular assays. The primary outcome reflecting clinical disease severity was length of hospital stay (LOS). A random-effects model was used to conduct the meta-analyses. RESULTS: Twenty-one studies involving 4,280 patients were included. The overall quality of evidence applying the GRADE approach ranged from moderate for oxygen requirements to low for all other outcomes. No significant differences in length of hospital stay (LOS) (mean difference (MD) −0.20 days, 95% CI −0.94, 0.53, p = 0.59), or mortality (RR 2.44, 95% CI 0.86, 6.91, p = 0.09) were documented in subjects with viral co-infections compared to those with a single viral infection. There was no evidence for differences in effects across age subgroups in post hoc analyses with the exception of the higher mortality in preschool children (RR 9.82, 95% CI 3.09, 31.20, p<0.001) with viral co-infection as compared to other age groups (I(2) for subgroup analysis 64%, p = 0.04). CONCLUSIONS: No differences in clinical disease severity between viral co-infections and single respiratory infections were documented. The suggested increased risk of mortality observed amongst children with viral co-infections requires further investigation.

Introduction:
Respiratory viral co-infections, defined as the detection of more than one viral pathogen in the same sample are detected in up to 30% of children with an acute respiratory tract infection (ARI) [1], [2]. While respiratory syncytial virus (RSV), influenza (INF) and human metapneumovirus (hMPV) have been mainly identified amongst children with single viral infections, other viruses including adenovirus (ADV), coronavirus and human rhinovirus (HRV) have been mainly reported amongst children with viral co-infections [2].
A better understanding of the effect of viral co-infections on disease severity is needed, considering the associated burden of respiratory viral infections. No systematic review on this topic has been published to date. A non-systematic and narrative review summarized eight cohort studies using either conventional techniques or molecular assays for detection of viruses [3]. The authors reported increased hospitalization rates among patients with viral co-infections compared to single respiratory viral infections (46.3% vs. 21.7%, p<0.01) suggesting increased severity with viral co-infections. These findings were limited, however, by including heterogeneous patient-populations (adults and children, with or without underlying comorbid conditions) and by combining studies using conventional as well as molecular methods for viral detection and by a potentially biased selection of the included studies.
We conducted a systematic review and meta-analysis to assess the association between viral status (i.e., single viral versus viral co-infection) as detected by molecular assays and the severity of clinical disease in children as well as adults hospitalized with an ARI. A systematic and comprehensive review of available literature was performed by using a transparent and systematic approach in searching, assessing and summarizing all the available evidence on this topic. We also aimed to explore whether heterogeneity in results was in part explained by different patient populations through subgroups analyses.

Eligibility criteria ::: Materials and Methods:
We included observational studies reporting on patients of any age admitted to hospital with an acute respiratory viral illness documented by molecular assays and comparing clinical disease severity between patients with viral co-infections to those with a single viral respiratory illness, as defined below. We excluded studies which only reported data on outpatients, used multiple diagnostic techniques for viral detection, or included viral-bacterial co-infections.

Primary and secondary outcomes ::: Materials and Methods:

The primary outcome reflecting clinical disease severity was length of hospital stay (LOS). This outcome is commonly used as a proxy of disease severity as it is thought to reflect well the duration of illness and is highly associated with health costs [5]. We selected a priori four secondary outcomes, which included admission to the intensive care unit, need for mechanical ventilation, oxygen requirements and mortality.

Literature search and data extraction ::: Materials and Methods:
We searched MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials, CINAHL and HTA (Health Technology Assessment) for relevant studies published any year or in any language up to January 28, 2013. The search strategy was created in collaboration with a librarian (Appendix S1 in File S1). We also searched conference proceedings from 2008 to 2013 using the Web of Science and Open-SIGLE databases, and we searched Clinical trial registries (clinicaltrials.gov) and Current Controlled Trials (controlled-trials.com). We also reviewed reference lists of key articles.
Two reviewers (M.E.S and S.A.A) independently screened the titles and abstracts. Potentially relevant full text articles were then screened independently for eligibility. Agreement was calculated using Cohen's kappa coefficient [6]. Disagreements were resolved by consensus discussion or third-party adjudication (A.M). Authors were contacted for additional information when required. Reviewers independently abstracted data using a standardized form.

Assessment of risk of bias and overall quality evidence ::: Materials and Methods:
Two reviewers who had content and methodological expertise independently and in duplicate assessed and graded the risk of bias for included studies with a previously used, adapted version [7] of the Newcastle-Ottawa scale (NOS), which has been described elsewhere [8]. The maximum score was 8, the minimum score 0. It was decided a priori that a score of 7 was reflective of high methodological quality (e.g., low risk of bias), a score of 5 or 6 indicated moderate quality and a score of 4 or less indicated low quality (e.g., high risk of bias). Assessment was conducted by both reviewers independently. Publication bias was assessed by visual inspection of funnel plot. The quality of evidence was independently assessed for each outcome by 2 reviewers according to the GRADE framework (Grading of Recommendations Assessment, Development and Evaluation) [9]. Disagreements were resolved as described above. Because randomized controlled trials addressing this research question are not feasible, observational studies were considered as the highest quality of evidence. The GRADE profiler (GRADEpro) [10] was used to present summary tables (Table 1).

Statistical analysis ::: Materials and Methods:
Statistical analysis was performed using Review Manager 5.1 (Cochrane Collaboration) [11]. Random-effects models were used to obtain summary estimates for all outcomes as heterogeneity across studies was expected. For continuous outcomes, we used the inverse variance method to combine results. If means and standard deviations were not reported and remained unavailable after contacting authors, the median was used to reflect the mean and the standard deviation calculated by dividing the interquartile range (IQR) by 1.35 [12]. For dichotomous outcomes, the risk ratio with its 95% confidence interval (CI) was reported. Heterogeneity was evaluated using the I^2 statistic.

Subgroup Analysis ::: Materials and Methods:
When substantial heterogeneity was found ($I^2 \geq 40\%$), a priori defined subgroup analyses were performed which included age (<18 years versus ≥18 years), the nature of the underlying respiratory illness (ARIs, bronchiolitis, radiologically confirmed community acquired pneumonia (CAP), respectively), the presence of underlying co-morbidities, the impact of overall viral infections versus specific viral pathogens, and high versus low risk of bias in the included studies as defined above. Interaction tests for subgroup differences were conducted using the Chi-square and I2 statistic. Subgroup credibility was examined using the criteria described by Sun and colleagues [13]. We made a post-hoc stratified analysis of “children” by age subgroups based on data availability in the abstracted studies (infants 0–23 months of age, preschool children 0–59 months of age and children 0–17 years old).

Sensitivity analysis ::: Materials and Methods:

We also evaluated the robustness of the pooled estimate related to the potential differences in disease severity described for infections with more pathogenic viruses such as INF, RSV or hMPV. Therefore we performed a post hoc sensitivity analysis in which only studies involving these viral infections were included [1],[2].

Study Characteristics ::: Results:
The 21 studies included reported on 4,280 patients ranging from 1 to 65 years of age (Table 2). Seventeen (80.9%) studies were exclusively conducted in children <18 years, two studies were conducted in adults and the remaining two in adults and children. Any ARI was assessed in nine studies; bronchiolitis, bronchitis and CAP in three studies each; and acute respiratory wheezing and influenza-like illness (ILI) in one study each. One study did not specify the type of respiratory illness.

Risk of Bias and Overall Quality of Evidence ::: Results:
Applying the adapted NOS, the studies achieved a median of 4 out of 8 points (IQR 3–4) (Table 3). The overall quality of evidence applying the GRADE approach ranged from moderate for oxygen requirements to low for all other outcomes (Table 1). One of the concerns involved serious indirectness for the LOS and the need for mechanical ventilation. For these outcomes, the majority of studies did not primarily focus on the comparison of LOS or the need for mechanical ventilation between single vs viral co-infections. A funnel plot did not suggest any publication bias for the primary outcome (Appendix S2 in File S1). Also, publication bias was judged to be minimal because of our extensive literature search, and the presence of both positive and negative studies.
Relevant subgroup analyses were conducted for the length of hospital stay and mortality as stated under these outcomes.

Primary outcome: length of hospital stay (LOS) ::: Results:
Eleven studies involving 2,531 patients reported the LOS and were pooled in meta-analysis. There was no significant difference in length of admission between groups (mean difference (MD) −0.20 days, 95% CI −0.94, 0.53, p = 0.59). (Figure 2) Because of significant heterogeneity (I2 = 73%, p<0.001), inconsistency and indirectness of the comparison documented for this outcome, the quality of evidence was downgraded to low.
Some of the heterogeneity could be explained by the type of respiratory illnesses: subgroup analyses showed a significantly shorter LOS stay among children with bronchiolitis and viral co-infection (MD −0.83 days; 95%CI −1.42, −0.24; p = 0.006) while no differences between viral groups were observed amongst subjects with ARI, although the interaction test was not significant (p = 0.10) (Appendix S3 in File S1). In post-hoc age subgroup analyses, the effect was similar across all age subgroups (I2 for subgroup analysis 0%, p = 0.77) (Figure 2).
Sensitivity analysis including only studies on viruses with a higher pathogenic potential (RSV A, RSV B, INF A, INF B, PIV and hMPV) revealed similar results (MD 0.24 days, −0.64, 1.12, p = 0.59) (Appendix S4 in File S1).

Secondary outcomes ::: Results:
Five studies provided data for meta-analysis on mortality including a total of 1,794 patients. There was a trend of higher mortality rates among patients with viral co-infections (RR 2.44, 95% CI 0.86, 6.91, p = 0.09) with a high degree of heterogeneity (I2 = 53%). Although there was a significant subgroup effect (I2 for subgroup analysis = 63%, p = 0.04) with higher mortality in preschool children with viral co-infections (RR 9.82, 95% CI 3.09–31.2, p<0.001), this was based on only one study, which was found to be at a high risk of bias (Figure 3).
Seven studies involving 893 patients reported the proportion of patients admitted to the ICU. No difference were observed (relative risk (RR) 0.72, 95% CI 0.40, 1.28, p = 0.26) with high heterogeneity (I2 = 53%). Also, there were no differences between age subgroups (I2 for subgroup analysis = 0%, p = 0.50) (Appendix S5 in File S1).
Only three studies including 515 patients provided detailed data on the need for mechanical ventilation. Again, there was no significant difference between groups (RR 1.58, 95% CI 0.61, 4.13, p = 0.35) but heterogeneity was high (I2 = 75%). Although there was a statistically significant difference in age group effects (I2 for subgroup analysis = 74%, p = 0.05), this was based on only two studies, one in infants with more benign outcomes (RR 1.01, 95% CI 0.57–1.78, p = 0.98) compared to one study in a mixed population (RR 2.69, 95% CI 1.22–5.94, p = 0.01) (Appendix S6 in File S1).

Eight studies involving 2,294 patients provided data for the meta-analysis on oxygen requirement as an outcome. No difference was found between both groups with high heterogeneity (RR 0.99, 95% CI 0.78, 1.26, p = 0.94, I2 = 47%). In post-hoc age subgroup analysis, the effect was similar across all age subgroups (for subgroup analysis = 48.5%, p = 0.12) (Appendix S7 in File S1).

Strengths and limitations ::: Discussion:
The strengths of this systematic review include a systematic, protocol-driven and comprehensive review with extensive literature search, minimal evidence for publication bias and successful attempts to contact authors. In addition, rigorous assessment of eligibility ensured high reliability of the results. All subgroups analyses, with the exception of age subgroups analyses, were defined a priori and post hoc sensitivity analyses confirmed robustness of the results. Finally a rigorous use of the GRADE approach ensured a transparent and comprehensive approach to evaluate overall quality of the studies. Limitations predominantly relate to the high risk of bias of the included studies. Of note, no studies reported risk estimates adjusted for important prognostic factors such as underlying co-morbidities or bacterial co-infections. Although the influence of comorbid conditions was partially addressed in our subgroup analysis, an insufficient number of studies precluded some subgroup analyses we planned a priori. Respiratory viral infections are known to predispose to secondary bacterial pulmonary infections, and thus can result in substantial confounding [30]. Finally, age subgroup analyses were conducted post hoc. Based on the data available we were able to provide subgroups as defined above, but the data did not allow us to specify other, better discriminating age groups.

Conclusions:
In conclusion, we found no convincing evidence that patients admitted with viral co-infections are at higher risk for increased disease severity than patients presenting with single respiratory viral infections –with the potential exception of mortality in preschool children of <5 years of age. Large, rigorously conducted studies including multivariable analyses adjusting for important confounders are lacking, and thus, new studies would be very likely to significantly change our overall assessment. Prospective longitudinal studies, which will focus on objective outcomes and include serial respiratory sampling for viruses and bacteria may lead to a better understanding of the clinical significance of polymicrobial acute respiratory infections.