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REGULAR ARTICLE

Virus detection in critically ill children with acute respiratory disease: a new profile in view of new technology

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ABSTRACT

Aim: To describe the epidemiology of critically ill children admitted to a paediatric intensive care unit (PICU) with acute respiratory disease. The association with intubation was analysed for the three most prevalent viruses and in those with and without viral co-infection.

Methods: Patients admitted to the PICU (2004–2014) with acute respiratory disease were included. Analyses were performed utilising each respiratory viral infection or multiple viral infections as an exposure.

Results: There were 1766 admissions with acute respiratory disease of which 1372 had respiratory virus testing and 748 had one or more viruses detected. The risk of intubation before or during the PICU stay was higher if parainfluenza virus was detected compared to respiratory syncytial virus (RSV) (OR: 2.20; 95% CI: 1.06–4.56). Sixty-three admissions had two or more viruses detected, and the combination of RSV and Rhinovirus/enterovirus was the most common. No significant difference was observed in the risk of intubation between patients with multiple and single viral infections.

Conclusion: Higher risk of intubation was found in patients with parainfluenza as compared to RSV. The risk of intubation comparing parainfluenza virus to other viruses and for patients with multiple versus single virus needs to be further studied.

INTRODUCTION

Acute respiratory diseases due to viral infections are common causes of illnesses in infants and young children around the world (1,2). The pathophysiology varies from upper airway obstruction (e.g. croup, laryngitis and laryngotracheitis) to severe lower respiratory tract illnesses (e.g. pneumonitis, bronchiolitis and acute respiratory distress syndrome).

The burden of respiratory viral infections in hospitalised paediatric populations has been well described. However, there are limited published data on the epidemiology and outcomes among critically ill children presenting with respiratory viral infections admitted to a paediatric intensive care unit (PICU) (3–5).

Abbreviation
DFA, Direct fluorescent antibody; HHHFNC, Heated humidified high-flow nasal cannula; hMPV, Human metapneumovirus; IQR, Interquartile range; LOS, Length of stay; OR, Odds Ratio; PCR, Polymerase chain reaction; PICU, Paediatric intensive care unit; PRISM, Pediatric risk of mortality; RSV, Respiratory syncytial virus.

Co-infection with multiple respiratory viruses is common in hospitalised children (6–8). Contrary to what one might predict, the severity of illness is not clearly greater with co-infection (6,7).

During the past decade, development and implementation of molecular technologies in the field of diagnostic virology have greatly enhanced our ability to detect respiratory viruses in clinical samples (9). For example, assays

Key Notes

- The data on burden of respiratory viral infection are limited, and there are conflicting findings on outcome of the multiple viral infections in a critical care population.
- Rhinovirus/enterovirus is the most common viral pathogen, and parainfluenza virus has the highest rate of requiring intubation in patients in paediatric intensive care unit with acute respiratory diseases.
- Further studies should be conducted to clarify the effects of multiple viral infections and their interactions in paediatric population.

using real-time reverse transcription polymerase chain reaction (RT-PCR) or respiratory pathogen panel using multiplex PCR for broad detection of viral pathogens have been incorporated into routine diagnostic tests in many laboratories (9–12).

It is important to delineate and understand the burden of respiratory viral infections and the differences in the outcomes as related to different respiratory viruses identified in critically ill children, to establish management strategies and target future research. In this study, we described the detection of respiratory viruses of a large cohort of children admitted to a single PICU with acute respiratory diseases over a ten-year period. We examined the association between endotracheal intubation before or during the PICU stay among the three most commonly identified respiratory viruses. We also compared the need for intubation between patients with multiple viruses versus those with only one respiratory virus.

MATERIALS AND METHODS

Setting and inclusion criteria

Stollery Children's Hospital PICU (patient age 0 to less than 17 years) is a western Canadian reference centre with a large catchment area, including Central and Northern Alberta, surrounding regions in Saskatchewan and British Columbia, as well as the Northwest Territories and Nunavut, with at least 750 000 children under 15 years of age (13). It is a mixed medical and surgical unit, with 800–1000 admissions per year over the study period, including 400–500 post cardiac surgery patients annually.

Admission demographics, diagnosis, severity of illness score (Pediatric Risk of Mortality Score III; PRISM III), treatments given during the PICU stay, and PICU and hospital outcomes from 2004 to 2014 (except 2009 because of lapse in data collection) have been collected in a unit-based administrative database (PICUEs®, WA, USA). Primary diagnoses in the database were categorised into 18 categories, and the secondary diagnoses coded using a binary scale with 21 categories (Appendices S1a and S2).

The inclusion criteria were patients admitted to the PICU 2004 through 2008 and 2010 through 2014: (1) with a primary or secondary diagnosis of 'asthma (reactive airway disease)' or 'pneumonia/bronchiolitis' or (2) with at least one of the search terms which indicated the presence of respiratory disease on admission (Appendix S1b) in the 'other (open label)' category of their primary or secondary diagnoses. Cases selected using the second criteria were included only if respiratory distress was the primary reason for PICU admission.

Respiratory virus testing and definition of prevalence

Detection of all respiratory viruses was performed at the Provincial Laboratory for Public Health. The temporal changes in respiratory virus testing methods and algorithms, thus the types of viral pathogens detected in our study periods and the platforms used to extract the testing data were described previously (14–16). Briefly, there were three periods with changes in respiratory virus targets and algorithms during the study period: (1) from 2004 to November 2005, respiratory syncytial virus (RSV), parainfluenza virus, influenza A and B and adenovirus were identified using direct fluorescent antibody (DFA) tests or rapid respiratory culture with mixed cell lines and DFA in a 24-well plate format, (2) from November 2005 to February 2008, rapid respiratory culture was replaced by in-house molecular diagnostic tests where human metapneumovirus (hMPV) was also detected, (3) since February 2008, the in-house molecular diagnostic tests were replaced by xTAG® RVP assay (Luminex Molecular Diagnostics Inc., Ontario, Canada) with Rhinovirus/enterovirus and four types of coronavirus as additional targets to the original six viruses. In addition, an in-house RT-PCR for influenza A and B with better sensitivity for pandemic influenza A was implemented in March 2009. Prior to March 2009, molecular diagnostic tests are performed on nasopharyngeal swabs that tested negative by DFA and all throat swabs and lower respiratory tract samples; since March 2009, in-house RT-PCR for influenza A and B +/− xTAG® RVP assay was performed on all sample types regardless of DFA results.

Respiratory virus testing results from five days before to five days after the date of admission to the PICU were included and analysed with each PICU admission counted as an independent event. With the changes in the respiratory virus testing algorithm over time, the number of admission events used as the denominator to calculate the overall and annual prevalence differed for various viruses (Table 1).

Table 1 Identified viral pathogens

Viruses	Number of cases	Prevalence*
Total admission	1766	
No test performed	392	222
All negative	624	353
*RSV**	274	155
Rhinovirus/enterovirus (Feb 2008-)	274	225
Parainfluenza virus	86	49
hMPV (Nov 2005-)	55	37
Adenovirus	50	28
Influenza A virus	37	21
Coronavirus (Feb 2008-)	21	17
Influenza B virus	16	9
Multiple infection		
RSV + Rhinovirus/enterovirus	23	19
RSV + Adenovirus	4	2
RSV + Influenza A/B virus	3	2
RSV + Parainfluenza virus	2	1
RSV + Coronavirus	2	2
RSV + hMPV	1	1
Rhinovirus/enterovirus + Parainfluenza virus	7	6
Rhinovirus/enterovirus + Coronavirus	4	3
Rhinovirus/enterovirus + hMPV	2	2

*Per 1000 admissions with respiratory distress.

Data analysis

Each continuous variable's distribution was described by median and interquartile range (IQR). Differences in treatments provided and outcomes for the PICU admissions, including PICU length of stay (LOS), hospital LOS, and PICU and hospital mortality, were also described for each of the viruses. After identifying the three most commonly detected viruses, logistic regression analyses utilising the viral detection as an exposure and adjusting for potential confounding variables, such as chronic diseases, prematurity and other conditions, the need of endotracheal intubation in the PICU, and endotracheal intubation before or during the PICU stay. Linear combinations of each parameter estimate were calculated with interaction terms for co-infections as multiple viral detections might have a synergistic effect on the outcomes. Linear regressions adjusting for the same set of potential confounding factors were performed to assess the effect of each virus on the log-transformed continuous outcomes as PICU LOS, hospital LOS and invasive ventilation days. Lastly, the effect of having more than one virus was examined. Mann–Whitney *U* test, Chi-Squared test and Fisher's exact test were used to compare the continuous and nominal demographics, and treatment variables as appropriate, between the two groups. Then, regression analyses adjusting for the same potential confounding variables were repeated to compare the outcomes between patients with a single virus and multiple viruses. A two-sided p-value of less than 0.05 was considered to be statistically significant. All statistical analyses were conducted with Stata version 13® (Stata Corp LP, Texas, USA). This study was approved by the Health Research Ethics Board of the University of Alberta.

RESULTS

Patients' demographics

There were 1766 admissions to the PICU that met our criteria for a primary acute respiratory disease during the study period; respiratory virus testing was performed for 1372 (78%). One or more respiratory viruses were detected among 748 admissions (42%) from children, with a median age of 1.1 years (IQR: 0.3–3.1 years). Sixty-eight per cent (N = 505) of these admissions had a primary or secondary diagnosis of pneumonia or bronchiolitis. For the 748 admissions, the median probability of death as predicted by PRISM III was 1.0%, and 18 (2%) of patients died during the PICU stay (Table S1). One hundred and seventy-one (10%) were the second or more repeated admissions during the primary single hospital admission in each given patient.

The three most prevalent respiratory viruses were as follows: Rhinovirus/enterovirus (N = 274; 225/1000 PICU admissions), RSV (N = 274; 155/1000 admissions) and parainfluenza virus (N = 86; 49/1000 admissions). The remaining viruses included hMPV (53, 37/1000 admissions), adenovirus (50, 28/1000 admissions), influenza A virus (37, 21/1000 admissions), 4 types of coronavirus (21, 16/1000 admissions) and influenza B virus (16, 9/1000 admissions). Two or more respiratory viruses were detected in 63 admissions (51/1000 admissions) (Table 1, Fig. S1). The hospital outcomes in the eight viruses were addressed in Table 2. Among all the viruses, adenovirus had a clinically significantly higher endotracheal intubation rate (36/50, 72%) and longer hospital LOS (19 days, IQR: 11–19).

Focusing on the three most prevalent viruses, the median age of PICU admissions with RSV (0.3 years, IQR: 0.1–1.4) was lower than Rhinovirus/enterovirus (1.3 years, IQR: 0.5–3.7) or parainfluenza virus (1.6 years, IQR: 0.7–4.1). Regarding the clinical presentation, apnoea was observed more frequently with RSV (11%) than the other two viruses. Symptoms of upper airway obstruction (i.e. croup, laryngotracheitis, laryngitis) were seen in 16% of the parainfluenza virus, which was significantly higher than RSV and Rhinovirus/enterovirus.

Comparison of management strategies and outcomes

Among the treatments provided in the PICU, there was variability in the use of steroids among Rhinovirus/enterovirus, RSV and parainfluenza virus. Heated humidified high-flow nasal cannula (HHHFNC) was also employed in different proportions. Although the overall mortality was low, admissions with the detection of Rhinovirus/enterovirus had significantly higher mortality as compared to RSV and parainfluenza (p = 0.03 and 0.01, respectively).

The probability of requiring endotracheal intubation before or during the PICU stay was significantly higher in admissions with parainfluenza virus as compared to RSV (OR: 2.20; 95% CI: 1.06–4.56); the absolute risks of endotracheal intubation were 129/274 (47%) in RSV, 118/274 (43%) in rhino/entero and 47/86 (55%) in parainfluenza, respectively (Table 3). Admissions with Rhinovirus/enterovirus detection had a slightly longer invasive ventilation days compared to the RSV (0.8 days; p = 0.02); there was a trend that PICU LOS and hospital LOS were longer in Rhinovirus/enterovirus compared to the RSV, except for hospital LOS in parainfluenza virus.

Effect of multiple viral infections

For the 63 PICU admissions where two or more respiratory viruses were detected, the most common combination was RSV and Rhinovirus/enterovirus (23 admissions, 19/1000 admission). The median age (0.7 years, IQR: 0.3–1.9) of the PICU admissions with more than two respiratory viruses detected was significantly lower than the admissions with a single virus. A larger proportion of admissions with multiple viruses had an admitting diagnosis of pneumonia/bronchiolitis (multiple viruses: 83% versus single virus: 66%, p = 0.008). Antibiotics were used more frequently on admissions with multiple viruses than those with single virus (97% vs. 88%, p = 0.034), whereas there was less steroid use in admissions with multiple viruses (33% vs. 47%, p = 0.043; Table S2). There was no significant difference in the risk of requiring endotracheal intubation before or during the PICU stay in the patients with multiple viruses versus single virus (1.89, 95% CI: 0.88–4.02) (Table 4). There was no difference in the number of invasive

Table 2 Patient outcomes for those with the eight viruses detected

Outcomes	Total admission n = 748	RSV n = 274	Rhino/entero n = 274	Para influenza n = 86	hMPV n = 55	Adeno n = 50	Influenza A n = 37	Corona n = 21	Influenza B n = 16
Intubation at PICU (%)	332 (44)	118 (43)	103 (38)	44 (51)	24 (44)	36 (72)	13 (35)	13 (62)	9 (56)
Intubation before or at PICU (%)	361 (48)	129 (47)	118 (43)	47 (55)	25 (45)	37 (74)	14 (38)	15 (71)	11 (69)
Invasive ventilation*†: Days (IQR)	6 (4–11)	7 (5–11)	6 (3–6)	5 (3–9)	6 (4–9)	7 (4–13)	6 (4–11)	6 (3–13)	5 (3–6)
PICU LOS*: Days (IQR)	4.4 (2.1–8.7)	4.8 (2.5–8.9)	3.8 (2.0–7.8)	3.5 (1.2–8.2)	5.8 (2.7–8.1)	5.9 (3.7–13.4)	4.9 (1.7–7.7)	5.5 (3.9–10.6)	5.5 (2.8–7.8)
Hospital LOS*: Days (IQR)	12 (7–24)	11 (7–17)	11 (6–26)	11 (7–28)	13 (8–23)	19 (11–46)	13 (7–28)	11 (8–37)	14 (7–22)
PICU death (%)	18 (2)	2 (1)	9 (3)	1 (1)	0 (0)	4 (8)	0 (0)	2 (10)	1 (6)
Hospital death (%)	23 (3)	2 (1)	11 (4)	1 (1)	0 (0)	6 (12)	0 (0)	2 (10)	2 (13)

IQR (interquartile range), *median of days, †for patients who was endotracheally intubated.

Table 3 Comparisons of risk of intubation in the three viruses

Viruses	Number of cases (%)	Adjusted ORs	95% CIs	p-Values
Intubation in PICU				
RSV	118 (43)	1.00 (Ref.)		
Rhinovirus/ enterovirus	103 (38)	1.09	0.63–1.87	0.77
Parainfluenza virus	44 (51)	1.76*	0.91–3.39	0.093
Intubation before or during PICU admission				
RSV	118 (43)	1.00 (Ref.)		
Rhinovirus/ enterovirus	129 (47)	1.20	0.65–2.21	0.56
Parainfluenza virus	47 (55)	2.20*	1.06–4.56	0.034

*PICU intubation, OR: 1.62 (95% CI: 0.81–3.22, p = 0.17) when comparing with Rhinovirus/enterovirus. Intubation before or at PICU, OR: 1.83 (95% CI: 0.85–3.93, p = 0.12) when comparing with Rhinovirus/enterovirus.

Table 4 Comparisons of risk of intubation and mortality: multiple viral infection versus single viral infection

Viruses	Number of cases (%)	Adjusted ORs	95% CIs	p-Values
Intubation in PICU*				
Single viral infection	305 (45)	1.00	0.47–2.02	0.94
Multiple infection	27 (43)	0.97		
Intubation before or during PICU admission*				
Single viral infection	327 (48)	1.00	0.88–4.02	0.10
Multiple infection	34 (54)	1.89		
PICU death				
Single viral infection	17 (2)			0.54†
Multiple infection	1 (2)			
Hospital death				
Single viral infection	22 (3)			0.41†
Multiple infection	1 (2)			

*Adjusted for confounding factors [i.e. age (days) at PICU admission, Vasopressor use (Y/N), Arterial Line use (Y/N), Central venous Line use (Y/N), Antibiotics use (Y/N), Steroid use (Y/N), PRISM (%), premature birth or chronic lung disease (Y/N), neuromuscular disease or neurological disorder (Y/N), Era: before February 2008 or after (Y/N)], †Univariate analyses with Fishers Exact tests.

ventilation days, PICU and hospital LOS, and mortality in the PICU and hospital stay between the two groups (Table 5).

DISCUSSION

There have been only a limited number of studies evaluating the epidemiology of respiratory viral infections in PICU population. Rhinovirus/enterovirus and RSV were the two most prevalent viruses in critically ill children admitted with acute respiratory disease in our PICU, which is similar to recent studies (17,18). Although there were minor annual fluctuations during the study period, the overall rate of detection of adenovirus, hMPV and other

Table 5 Comparisons of other outcomes: multiple viral infection versus single viral infection

	Medians (IQRs)	Adjusted differences	p-Values
Invasive ventilation days: Days*			
Single viral infection	6 (4–11)	Ref.	0.86
Multiple infection	7 (4–9)	1	
PICU LOS: Days*			
Single viral infection	4.4 (2.1–8.7)	Ref.	0.86
Multiple infection	4.9 (3.0–8.7)	1.0	
Hospital LOS: Days*			
Single viral infection	12 (7–25)	Ref.	0.69
Multiple infection	11 (8–20)	1	

*Adjusted for confounding factors [i.e. age (days) at PICU admission, Vasopressor use (Y/N), Arterial Line use (Y/N), Central venous Line use (Y/N), Antibiotics use (Y/N), Steroid use (Y/N), PRISM (%), premature birth or chronic lung disease (Y/N), neuromuscular disease or neurological disorder (Y/N), Era: before February 2008 or after (Y/N)].

respiratory viruses was not as high as compared to published studies of non-PICU settings (7,19–22). The potential reasons could be related to our inclusion criteria, i.e. all critically ill children with acute respiratory disease. It is worth noting that 2009, the year with the emergence of pandemic H1N1 influenza, was excluded from our analysis due to missing data, which provided better analyses and comparison of respiratory virus activities for the nonpandemic years.

When comparing the clinical management of the different respiratory viruses, the risk of intubation for admissions with parainfluenza virus was significantly higher than the admissions with RSV and Rhinovirus/enterovirus. There have been only a few studies looking at the impact of parainfluenza virus infection in the critically ill children (23,24). Given that the severity of illness did not differ significantly among the three viruses at the time of the PICU admission, specific pathogenicity of each virus such as the higher likelihood of laryngotracheitis with parainfluenza virus infections might have led to the higher rate of endotracheal intubation. There was a significant increase in the number of invasive ventilation days with Rhinovirus/enterovirus versus RSV. Our finding was discordant from a previous study with a smaller-hospitalised paediatric cohort, but the difference in invasive ventilation days was only 0.8 day in our cohort (25).

The mortality rate was highest in PICU admissions with detection of Rhinovirus/enterovirus. It is important to note that the detection of Rhinovirus/enterovirus only started in November 2008 with the use of the xTAG® RVP assay, so there is the possibility of misclassified cases from 2004 to November 2008. Differentiation between Rhinovirus and enterovirus is performed only by request from the clinicians. Thus a positive Rhinovirus/enterovirus result includes a broad range of viruses, which generally causes mild upper respiratory infections in healthy populations. A retrospective cohort study which includes all patients admitted to PICUs with respiratory illness and Rhinovirus/enterovirus at three urban centres reported a mortality rate of 2.1% (11/519) which was similar to our findings of 3.3% (18). With the retrospective design of our study, it was difficult to delineate the immediate or direct cause of death, which could be related to underlying disorders predisposing the children to be sicker with a concomitant viral infection.

We observed a trend that PICU admissions with more than two respiratory viruses were associated with a higher rate of requiring endotracheal intubation before or during the PICU stay although this was not statistically significant; it should be also noted that the disease severity given by the PRISM III was similar between admissions with single and multiple viruses detections. Our ability to identify mixed virus infection was limited before the use of molecular diagnostic tests in November 2005, and some cases with a predominant virus detected by DFA from their nasopharyngeal swabs might not have molecular diagnostic tests performed prior to March 2009 if no throat swabs or lower respiratory samples were submitted for testing. Another limitation is that PRISM III only represented the initial assessment at PICU admission. The higher rate of antibiotic use in admissions with multiple viruses might suggest that more of these patients presented to the PICU with a more severe clinical picture; nonetheless, the exact antibiotic indications could not be obtained from our database. A recently performed systematic review concluded that there was no difference in clinical disease severity between single infection and co-infections of respiratory viruses; however, in their subgroup analysis, the authors found a higher mortality in preschool children with viral co-infection as compared to other age groups (6). A small cohort study reported that infants with severe bronchiolitis due to co-infections had higher odds to be admitted to the PICU than did those with a single virus; as in our study, the most dominant combination of the viruses was RSV and Rhinovirus (26). A single centre-study investigating the effect of viral co-infection in a two-year retrospective study, in which the diagnostic panel did not include Rhinovirus/enterovirus, reported that the most common co-infections were with adenovirus and RSV and found a significantly higher severity in patients with viral co-infections (7). However, no difference in the risk of invasive ventilation requirement and ICU LOS was observed. The difference in findings among those studies might be related to the difference in demographic factors such as age and underlying co-morbidities.

There are several limitations to our study. Admission events with respiratory disease were selected based on a retrospective review of PICU admission diagnosis; thus, the population is likely to be heterogeneous, with a risk of some patients being misclassified. Some misclassifications would have occurred with the changes in respiratory virus testing algorithm overtime and the fact that not all patients were subjected to the same molecular diagnostic tests should be noted. A small number of patients

might have multiple PICU admissions due to the same illness; each new admission was counted as a separate event in the analyses. The detection of a viral pathogen around the time of admission does not necessarily represent a causal relationship with the respiratory symptoms as some of the virus detected on the PICU admission could be a result of chronic viral shedding from a prior infection. A study reported that only Rhinovirus-A and Rhinovirus-C were associated with acute respiratory illness, hospitalisation, and subsequent serious illness among the subtypes (27). However, an enterovirus containing many species of virus from the same *picornaviridae* family was grouped together by the diagnostic test in our study. Similarly, RSV A versus RSV B, parainfluenza virus 1–4 and various adenoviruses, influenza A and B viral strains were not differentiated. Other important contributors to disease severity such as secondary or concomitant bacterial infection, existing immune-compromised condition, were not considered in the data analysis (28,29). To minimise this potential bias, antibiotic usage was used as a proxy, but some of the antibiotics would have been started just as empiric treatment. Finally, even though our study had one of the largest cohorts in the PICU setting, it was underpowered to evaluate the specific effects of interactions among the various viruses. It is possible that only certain combinations of co-infections are associated with worse outcomes. A case-control study of hospitalised children with acute respiratory infection in Vietnam showed 9.5% of the cohort had multiple viral infections (8), with RSV increasing the risk of hospitalisation when co-infected with Rhinovirus, hMPV and parainfluenza virus, but not with influenza A virus. These types of interactions should be further investigated in specific populations such as PICU patients in a multicentre study with a larger sample size.

CONCLUSIONS

Rhinovirus/enterovirus was the most common respiratory viral pathogen detected in a large cohort of children presented with acute respiratory disease during admissions to a PICU, surpassing RSV as the main culprit. The risk of endotracheal intubation in the PICU and before or during the PICU stay was higher for admissions with parainfluenza virus detection as compared to RSV and Rhinovirus/enterovirus; however, there was no significant difference in the risk of needing endotracheal intubation before or during the PICU stay in the patients with multiple viruses versus single virus. Further studies should be conducted to clarify the effects of multiple viral infections, their interactions and the role of bacterial co-infection in paediatric population.

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CONFLICT OF INTERESTS

None of the authors have any relevant conflict of interest to declare.

CLINICAL TRIAL REGISTRATION

None.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 The trend of proportions in eight virus groups.
Table S1 Patient demographics for those with the eight respiratory viruses detected.
Table S2 Comparisons of demographics and outcomes: multiple viral infection versus single viral infection.
Appendix S1a Primary diagnosis.
Appendix S1b Search terms for 'Others'.
Appendix S2 Secondary diagnosis.

