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Original article

Myxovirus resistance protein A for discriminating between viral and bacterial lower respiratory tract infections in children – The TREND study

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ABSTRACT

Objective: Discriminating between viral and bacterial lower respiratory tract infection (LRTI) in children is challenging, leading to an excessive use of antibiotics. Myxovirus resistance protein A (MxA) is a promising biomarker for viral infections. The primary aim of the study was to assess differences in blood MxA levels between children with viral and bacterial LRTI. Secondary aims were to assess differences in blood MxA levels between children with viral LRTI and asymptomatic controls and to assess MxA levels in relation to different respiratory viruses.

Methods: Children with LRTI were enrolled as cases at Sachs' Children and Youth Hospital, Stockholm, Sweden. Nasopharyngeal aspirates and blood samples for analysis of viral PCR, MxA, and C-reactive protein were systematically collected from all study subjects in addition to standard laboratory/radiology assessment. Aetiology was defined according to an algorithm based on laboratory and radiological findings. Asymptomatic children with minor surgical disease were enrolled as controls.

Results: MxA levels were higher in children with viral LRTI ($n = 242$) as compared to both bacterial ($n = 5$) LRTI ($p < 0.01$, area under the curve (AUC) 0.90, 95% CI: 0.81 to 0.99), and controls (AUC 0.92, 95% CI: 0.88 to 0.95). In the subgroup of children with pneumonia diagnosis, a cutoff of MxA 430 μg/l discriminated between viral ($n = 29$) and bacterial ($n = 4$) aetiology with 93% (95% CI: 78–99%) sensitivity and 100% (95% CI: 51–100%) specificity (AUC 0.98, 95% CI: 0.94 to 1.00). The highest MxA levels were seen in cases PCR positive for influenza (median MxA 1699 μg/l, interquartile range: 732 to 2996) and respiratory syncytial virus (median MxA 1115 μg/l, interquartile range: 679 to 2489).

Discussion: MxA accurately discriminated between viral and bacterial aetiology in children with LRTI, particularly in the group of children with pneumonia diagnosis, but the number of children with bacterial LRTI was low. **Samuel Rhedin, Clin Microbiol Infect 2022;28:1251**

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Introduction

The roll-out of pneumococcal conjugate vaccines has shifted the aetiology of lower respiratory tract infections (LRTIs) in children from bacterial to viral aetiology [1–3]. However, distinguishing viral from bacterial LRTI is challenging, and many children with viral LRTI receive unnecessary antibiotic treatments, which drives antimicrobial resistance [4]. In children with severe bacterial LRTI, antibiotic treatment has a significant impact on the outcome [5]. There is a need for new biomarkers that better distinguish viral from antibiotic-requiring bacterial LRTIs for an improved use of antibiotics [6].

Myxovirus resistance protein A (MxA) is a promising biomarker for viral infections that is produced in a variety of immune cells as a response to interferon signaling [7–9]. MxA has also been shown to be helpful for distinguishing between acute respiratory infection and asymptomatic detection and to guide patient flows in the emergency ward [8,10–12].

The primary aim of the study was to assess the difference in blood MxA levels between cases with viral and bacterial LRTI. Secondary aims were to assess differences in blood MxA levels between children with viral LRTI and asymptomatic controls and in relation to respiratory pathogens.

Methods

Study design and population

The study protocol for the Trial of Respiratory infections in children for Enhanced Diagnostics (TREND) study was registered at clinicaltrials.org on July 28, 2017 (id: NCT03233516) and has previously been published [13]. Briefly, children 1 to 59 months of age with clinical pneumonia were prospectively enrolled at the emergency department at Sachs' Children and Youth Hospital, Stockholm, Sweden, November 2017 to December 2019. Clinical pneumonia was defined according to WHO as 1) cough or breathing difficulties and 2) tachypnea ($\geq$50 breaths/minute in children <12 months and $\geq$40 breaths/minute in children >12 months) or lower chest indrawing. Children with auscultatory rhonchi and chest indrawing underwent bronchodilator challenge (BDC) as previously established [14]. The BDC was considered positive if the chest indrawings had resolved upon re-evaluation. As few children were diagnosed with pneumonia, we chose to use the term LRTI to describe the condition of the cases. Children with minor surgical/orthopaedic disease with no reported respiratory symptoms during the previous 7 days or hospitalization during the previous 14 days were enrolled as controls. Parents of control subjects were contacted <2 weeks after enrollment and were interviewed regarding any newly developed respiratory symptoms. Written informed consent was collected from the caregivers of all study subjects. The study was approved by the Regional Ethics Review Board in Stockholm (dnr 2017/958-31).

Sampling and data collection

A nasopharyngeal aspirate was collected from all the study subjects (Table S1). A blood test collected by finger prick into a capillary tube was obtained for the analysis of MxA and C-reactive protein (CRP) (cases only). Cases underwent a standardized examination and background characteristics were collected using an electronical questionnaire. Data on management and diagnostics were collected from the medical records. Treatment failure was defined as a new antibiotic prescription or a new related revisit <30 days.

Microbiological and biochemical analyes

CRP was analysed using the Alere Afinion AS100 Analyzer. For the MxA analyses, 20 μl of capillary blood was collected using a heparinized capillary tube and diluted in a hypotonic buffer. Diluted samples were stored frozen at −70°C and transported batchwise with dry ice to University of Turku, Finland, where they were analysed using an enzyme immunoassay [8]. The assay measures MxA levels against a recombinant human MxA standard produced in insect cells using baculovirus expression system. Interassay variation of the study runs was 18%. The nasopharyngeal aspirates were analysed by multiplex PCR for influenza A/B, respiratory syncytial virus (RSV), adenovirus, bocavirus, coronaviruses (HKU1, NL63, OC43, and 229E), human metapneumovirus (hMPV), parainfluenza virus (PIV) 1-3, rhinovirus, enterovirus, *Streptococcus pneumoniae*, *Haemophilus influenzae, Bordetella pertussis*, *Mycoplasma pneumoniae and Chlamydophila pneumoniae*) at Sahlgrenska University Hospital, Gothenburg, as previously described [15].

Statistical analyses

Aetiology was defined based on radiographic and microbiological findings and CRP levels according to the *a priori* defined TREND aetiology algorithm (Online Supplement) [13]. Area under the curve (AUC) and 95% CIs for MxA to predict viral LRTI were calculated using receiver operating characteristic (ROC) curves comparing viral versus bacterial LRTI as well as viral LRTI versus controls. Optimal cutoffs for MxA were determined using the Youden index. The Mann-Whitney U-test and Kruskal-Wallis nonparametric tests were used for the comparisons of MxA levels between groups as appropriate. We aimed at including a total of 300 cases and 120 controls to obtain a sufficient number of cases with bacterial LRTI for the main analyses of MxA [13]. Nevertheless, the inclusion of controls was less successful than expected and the number of cases with bacterial LRTI lower than the estimated prevalence based on a previous pneumonia study [3].

Different sensitivity analyses were performed. First, analyses were carried out after exclusion of cases with positive BDC. Second, analyses were restricted to children who were diagnosed with pneumonia. When comparing children with viral LRTI and controls, MxA levels were assessed in the subgroups of a) viral PCR positive controls and b) viral PCR negative controls that had not developed respiratory symptoms at follow-up (defined as strict controls). Finally, we assessed the difference in MxA levels between the groups specified above using a lower CRP cutoff (60 mg/l) for the classification of bacterial aetiology as previously suggested by Nijman et al. [16]. Data analysis was performed in Stata version 16.1 (StataCorp, College Station, TX, USA) and GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, CA, USA).

Results

After exclusion of one case with missing MxA data, a total of 326 cases and 47 controls were included. A total of 242 cases were classified as having viral LRTI, 11 cases had mixed viral–bacterial LRTI, 5 cases had bacterial LRTI, 2 cases had atypical bacterial LRTI, and 66 cases had LRTI of undetermined aetiology. A total of 107/326 (33%) cases were admitted to an inpatient ward; in 77/326 (24%) cases a chest x-ray was performed, and 50/326 (13%) cases were diagnosed with pneumonia. Antibiotics were prescribed in 91/324 cases (28%), in 60/242 (25%) of cases with viral aetiology and in 4/5 (80%) of cases with bacteral aetiology. The most commonly identified viruses in the cases were rhinovirus, RSV, and hMPV (detected in $n = 156$, $n = 125$, and $n = 32$ cases, respectively) [17]. Of the children with viral LRTI who were treated with antibiotics, 21/

60 (35%) showed signs of treatment failure. Of the 11 children with mixed viral–bacterial LRTI, 5 had radiographic evidence of lobar pneumonia and the median CRP was 123 mg/l. The most common viral PCR findings were RSV, influenza, and hMPV (detected in $n = 5$, $n = 4$, and $n = 3$ cases with mixed viral–bacterial LRTI, respectively).

Among the controls, 24/47 (51%) tested positive for one or more respiratory viruses by PCR, and 14/47 (30%) developed respiratory symptoms during the follow-up period.

Cases were younger than controls (p <0.001), less likely to attend daycare (p <0.01), and more likely to suffer from a chronic disease (p = 0.04) (Table 1).

MxA for the differentiation between viral and bacterial LRTI

The highest MxA levels were seen in children with mixed viral–bacterial LRTI (median 1043 μg/l, IQR: 685–2996 μg/l) and viral LRTI (median 777 μg/l, IQR: 406–1659 μg/l) (Fig. 1). MxA levels were significantly higher in the children with viral LRTI as compared to bacterial LRTI (median MxA 777 μg/l vs 145 μg/l, respectively, p <0.01). ROC curves for viral versus bacterial aetiology showed an AUC of 0.90, 95% CI: 0.81 to 0.99 (Fig. 2A). The optimal cutoff for MxA was 430 μg/l, yielding a specificity of 100% (95% CI: 57–100%), and a sensitivity of 72% (95% CI: 66–78%) (Fig. 2A). The AUC was 0.92 (95% CI: 0.82–1.00) when excluding children with positive bronchodilator challenge comparing viral with bacterial aetiology (Fig. 2B). Finally, in the analysis restricted to children with pneumonia, the AUC was 0.98 (95% CI: 0.94–1.00), comparing cases with viral ($n = 29$) and bacterial ($n = 5$) aetiology (Fig. 2C). In this analysis, a cutoff of MxA 430 μg/l resulted in 100% (95% CI: 51–100%) specificity and 93% (95% CI: 78–99%) sensitivity for the identification of viral aetiology.

In a sensitivity analysis the CRP cutoff for defining bacterial aetiology was lowered to 60 mg/l. This resulted in an AUC of 0.82 in the full cohort and AUC 0.94 in children with pneumonia diagnosis (Table S2).

Differences in MxA levels between viral LRTI and controls

MxA levels were higher in cases with viral LRTI as compared to controls (median MxA 777 μg/l and 85 μg/l respectively, p <0.001) (Fig. 3A). There was no statistically significant difference in MxA levels between PCR positive ($n = 24$) and PCR negative ($n = 23$) controls (median MxA 103 μg/l vs 71 μg/l respectively, p = 0.57).

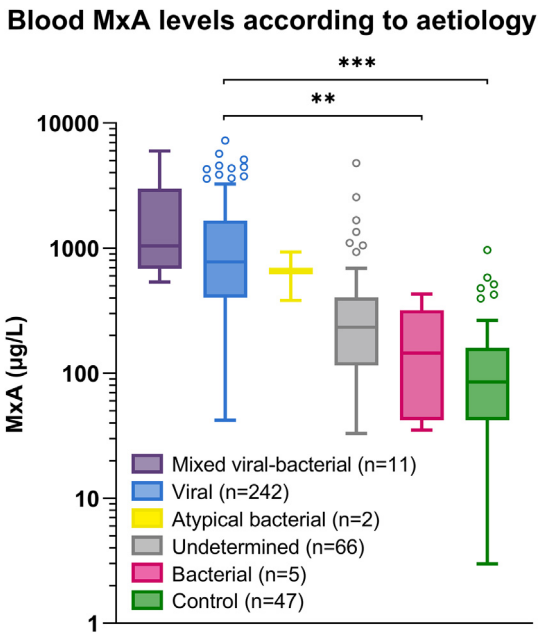

Fig. 1. MxA levels in children with lower respiratory tract infection and asymptomatic controls according to aetiology. Boxplot of blood MxA levels in children with lower respiratory tract infections, stratified by aetiology, and in asymptomatic controls (green). Boxes indicate median and IQR. Whiskers indicate 1.5 × interquartile range. P values calculated with Mann-Whitney U-test. **p <0.01; ***p <0.001. MxA, Myxovirus resistance protein A.

The ROC curve for the discrimination between viral LRTI and controls resulted in an AUC of 0.92, (95% CI: 0.88–0.96), which increased to 0.96 (95% CI: 0.93–0.99) when excluding cases with positive BDC and comparing with the strict controls (Fig. 3B). Further, cases were compared to PCR positive controls, after exclusion of those with positive BDC, which decreased AUC to 0.91 (95% CI: 0.85–0.97) (Fig. 3C). Finally, the AUC was 1.00 (95% CI: 0.99–1.00) in the discrimination of cases with viral physician-diagnosed pneumonia from the strict controls (Fig. 3D). In the analysis of MxA levels in viral pneumonia compared to strict controls, the optimal MxA cutoff was 264 μg/l, corresponding to 100% specificity and 97% sensitivity. When the CRP cutoff for defining bacterial aetiology was lowered to 60 mg/l the AUC was 0.91 in the full cohort and AUC 1.00 when comparing children with viral pneumonia compared to the strict controls (Table S2).

Table 1
Sociodemographic characteristics of study participants

Characteristic	Viral LRTI ($n = 242$)	Bacterial LRTI ($n = 5$)	All cases ($n = 326$)	Controls ($n = 47$)	p value[a]
Age (months), median IQR	11.3 (4.8–18.5)	22.3 (21.6–26.9)	13.1 (5.6–21.7)	25.5 (12.7–42.0)	**<0.001**
1–11 months	*125 (52)*	*1 (20)*	*152 (47)*	*10 (21)*	**<0.001**
12–59 months	*117 (48)*	*4 (80)*	*174 (53)*	*37 (79)*	
Male sex	149 (62)	1 (20)	200 (61)	22 (47)	0.06
Attending daycare	92 (39)	4 (80)	135 (42)	29 (64)	**<0.01**
Breastfeeding	82 (34)	—	105 (33)	12 (26)	0.37
Parental smoking	36 (15)	1 (20)	45 (14)	5 (11)	0.57
Fully immunized[b]	237 (99)	5 (100)	319 (99)	45 (98)	0.61
Chronic disease	72 (30)	3 (60)	105 (32)	8 (17)	**0.04**
Asthma/wheezing	*68 (28)*	*3 (60)*	*100 (31)*	*8 (17)*	*0.06*
Other[c]	*4 (2)*	-	*9 (3)*	*1 (2)*	*1.0*
University studies ≥1 parent	175 (72)	5 (100)	246 (76)	33 (70)	0.44

LRTI, lower respiratory tract infection; IQR, interquartile range.
Bold indicates significant p-values.
[a] Comparing cases with controls.
[b] According to age.
[c] Chronic heart/lung disease ($n = 6$), congenital syndrome ($n = 3$), neurological disease ($n = 2$), other disease ($n = 8$).

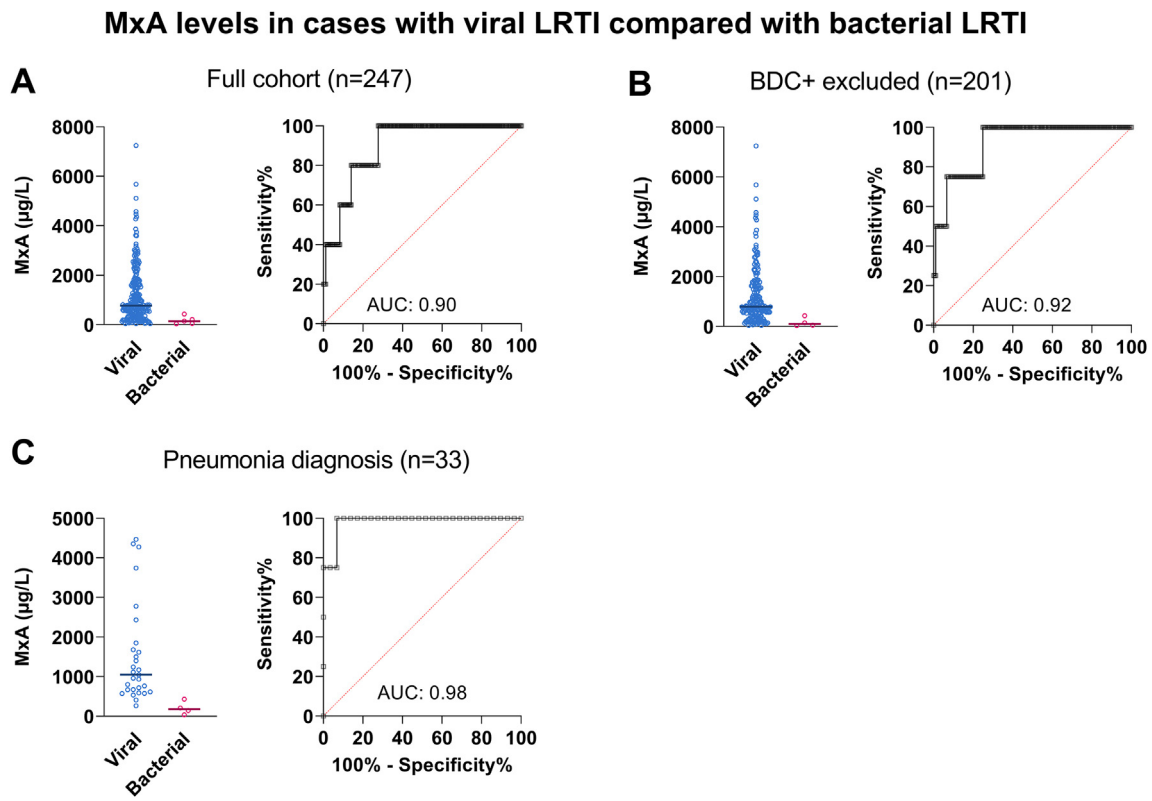

Fig. 2. MxA levels in children with viral and bacterial LRTI. Blood MxA-levels and receiver operating characteristic curves comparing children with viral (blue) and bacterial (pink) LRTI in (A) the full cohort, (B) after exclusion of cases with positive bronchodilator challenge, and (C) restricted to cases with pneumonia diagnosis. AUC, area under the curve; BDC, bronchodilator challenge; LRTI, lower respiratory tract infection; MxA, myxovirus resistance protein A.

MxA-levels according to detected virus and age

The highest MxA levels were seen in cases with a single infection of adenovirus (median 1961 µg/l, IQR: 924–3994), RSV (median 1226 µg/l, IQR: 747–1834), PIV (median 985 µg/l, IQR: 728–2146), influenza (median 783 µg/l, IQR: 647–1244) and hMPV (median 759 µg/l, IQR: 567–1616) (Fig. 4A). When considering all viral detections (single infections and coinfections of two or more viruses), the highest MxA levels were seen in cases positive for influenza (median 1699 µg/l, IQR: 732–1996), RSV (median 1173 µg/l, IQR: 693–2283), and hMPV (median 1115 µg/l, IQR: 679–2489) and there was less of a difference between the viruses (Fig. 4B). The MxA levels were higher in cases 1 to 12 months old as compared to children 1 to 4 years, both when only considering cases with viral single infections and when considering all cases testing positive for one or more virus ($p = 0.016$ and $p = 0.001$, respectively) (Fig. S1).

Discussion

In this prospective study of children with LRTI, we report that MxA accurately discriminates between viral and bacterial aetiology. Previous studies of MxA in children have reported a strong correlation with viral symptomatic infections [7,8,11]. Engelmann et al. assessed MxA levels in children with viral respiratory or gastrointestinal infections compared to children with microbiologically confirmed bacterial infections and reported accuracy similar to our main analysis (AUC 0.89 as compared to 0.90 in our study) [7]. The calculated optimal cutoff for MxA to discriminate between viral and bacterial aetiology in our study was similar to that (434 µg/l) of a study in children with febrile urinary tract infections with or without concomitant respiratory viral infection [9]. In other studies comparing viral and bacterial infections, the optimal cutoff has been lower (40–200 µg/l) [7,8,18]. Assay standardization, study population, and pathogens detected may have impacted results, making direct comparison of concentrations between different studies difficult [7,11].

We found MxA to be highly accurate in discriminating between viral and bacterial aetiology, particularly in children with physician-diagnosed pneumonia (AUC >0.98). We also assessed MxA levels according to detected virus and found the highest MxA levels in children postive for adenovirus, RSV, PIV, influenza, and metapneumovirus, i.e., the viruses that were considered as the most relevant PCR findings in the aetiology algorithm [2,3,19]. This is also in line with a previous study by Toivonen et al. and harmonizes with the clinical conception of the pathogenicity of these viruses [8]. Variable MxA levels in rhinovirus infections seem to reflect mild infections or sporadic detections where the virus is a bystander in a mixed infection. There were only two cases with atypical bacterial LRTI (positive for *M. pneumoniae*) but both had elevated MxA (>350 µg/l).

There are other promising biomarkers for LRTI in children, the most studied being a combination test of CRP, interferon gamma induced protein-10, and tumor necrosis factor-related apoptosis-inducing ligand [20,21]. In a multicenter study of preschool children with LRTI or febrile illness of unknown origin, this test was superior to procalcitonin in the distinction between viral and bacterial aetiology (AUC 0.90 in the full cohort and AUC 0.92 in cases with unanimous panel diagnosis) [22]. They assigned aetiology based on an expert panel whereas we used an

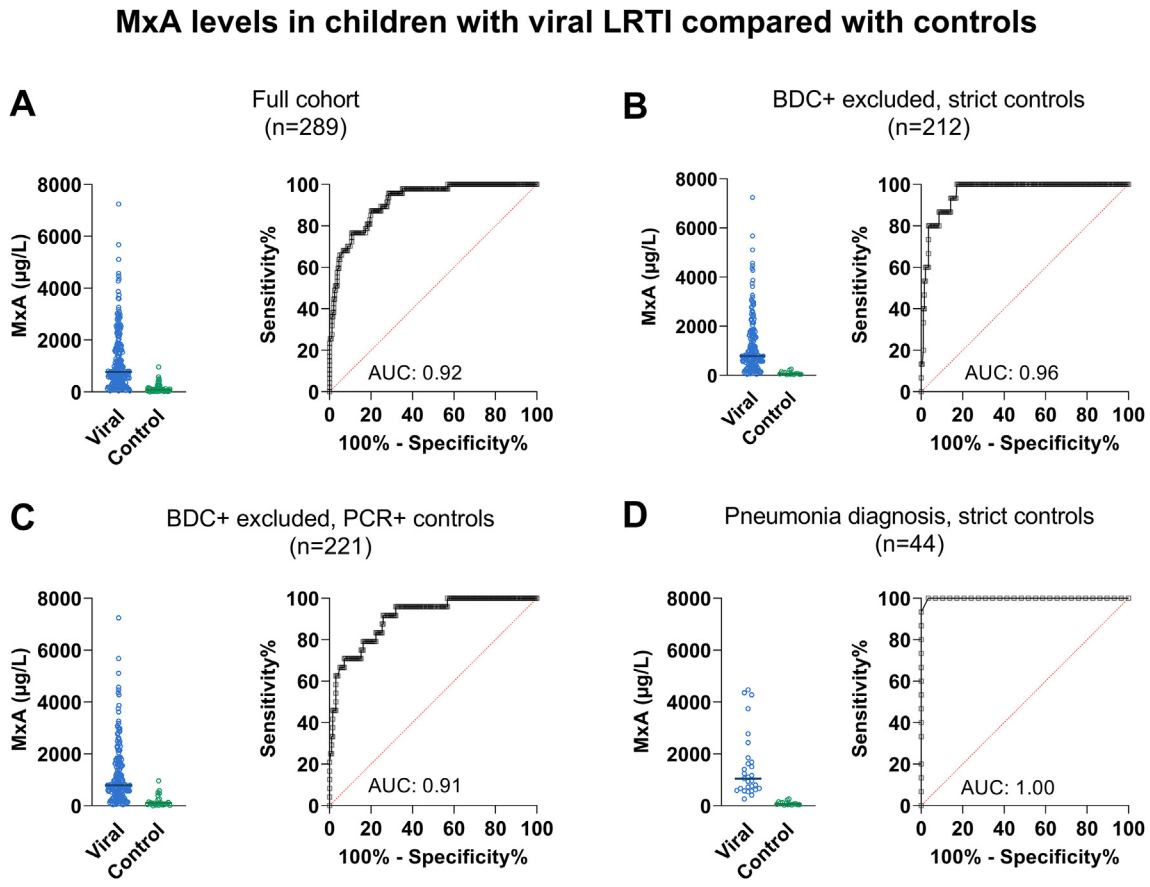

Fig. 3. MxA levels in children with viral LRTI compared to asymptomatic controls. Bood MxA levels and receiver operating characteristic curves comparing children with viral lower respiratory tract infection (blue) and controls (green) in (A) the full cohort, (B,C) after exclusion of cases with positive BDC compared to (B) strict controls ($n = 15$) (i.e., excluding controls testing positive by PCR for any respiratory virus or reporting respiratory symptoms at follow-up) or compared to (C) PCR + controls. (D) comparison of cases with pneumonia diagnosis and strict controls (as defined in B). AUC, area under the curve; BDC, bronchodilator challenge; LRTI, lower respiratory tract infection; MxA, Myxovirus resistance protein.

algorithm [13,22]. Nevertheless, both approaches partly rely on the imperfect biomarker CRP, which has significant limitations in diagnosing causes of paediatric infectious diseases [6].

The use of transcriptomic methods is another interesting approach in the development of future biomarkers [23–25]. Compared to methods assessing gene expression, measurement of MxA protein levels in the blood may have advantages such as predictable kinetics and straightforward analytic technology that can be adapted to existing laboratory processes.

While most biomarker research is performed in high-income countries, the burden of disease due to LRTI is highest in low- and middle-income countries and the impact of new biomarker-guided antimicrobial stewardship practice is even more important in these settings [26,27].

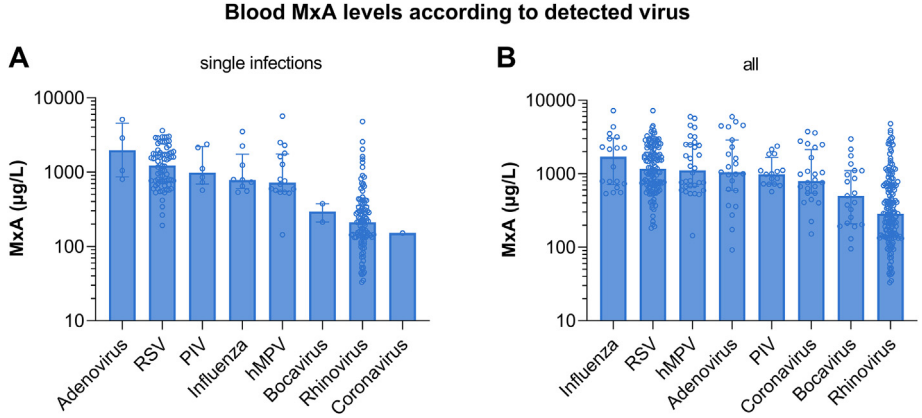

Fig. 4. MxA levels according to detected virus. Bar plot of median MxA levels according to detected virus in (A) cases with viral single infection and (B) all virus-positive cases. Bars for coronavirus, influenza virus, parainfluenza virus represent all subtypes of the viruses. Bars indicate median, whiskers indicate interquartile range. hMPV, human metapneumovirus; MxA, myxovirus resistance protein A; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Strengths of the current study include the *a priori* defined aetiology algorithm and the prospective study design. The study also has some limitations. The few children with bacterial LRTI resulted in low statistical accuracy. This was partly due to the fact that we used the WHO criteria as case definition, which has poor accuracy for identifying bacterial LRTI and also includes viral-induced wheeze [28]. We believe, however, that the cohort represents a distinct and large proportion of children seeking care at pediatric emergency departments and that our results are generalizable to similar high-income country settings.

Further, as recent studies have suggested lower CRP thresholds for the identification of bacterial infections, we performed a sensitivity analysis with a lowered CRP cutoff (60 mg/l) for assigning bacterial aetiology and still observed good accuracy for MxA, in particular for the group of children with pneumonia diagnosis [16,29].

Finally, we did not assess children who were classified as having mixed viral–bacterial aetiology. These children had both high MxA levels and high CRP or consolidations on chest x-rays and they were largely treated with antibiotics. Although some of these children likely had solely viral infections, they are not prioritized for recommendations to withhold antibiotics and in previous biomarker studies, children with unclear or mixed viral–bacterial aetiology have been excluded [22,30]. Data suggest that mixed viral–bacterial infections are likely underestimated and hence we feel that it may be an oversimplification to dichotomize childhood pneumonia into either bacterial or viral in aetiology [1,3]. From a clinical perspective, it might be more relevant to discriminate between antibiotic-requiring and self-limiting LRTI and ultimately, we believe that the safety of a biomarker-guided antibiotic treatment strategy should be evaluated in a randomized controlled trial.

To conclude, MxA accurately discriminated between viral and bacterial aetiology in children with LRTI, in particular in the group of children with pneumonia but the number of cases with bacterial LRTI was low. This study provides further evidence of MxA being a biomarker with high specificity for viral infection that could serve as a useful component in a combination test with other biomarkers.

Transparency declaration

M.R.R. reports consulting fees from Janssen Pharmaceutica as coordinating investigator for clinical trial on novel therapeutics for respiratory syncytial virus. M.W. reports grants from Jenny and Antti Wihuri Foundation, grants from Turku University Hospital Foundation during the conduct of the study, and from Labmaster Ltd, outside the submitted work. The other authors have no conflicts of interest to disclose.

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Deidentified participant data can be provided upon requests if providing a reasonable proposal once an appropriate data sharing agreement has been established with Karolinska Institutet. The study protocol is available at clinicaltrials.org (id:NCT03233516).

Author contributions

S.R. conceptualized the study, had a leading role in the study design, monitored the data management and recruitment of study subjects, performed the statistical analyses, drafted the first version of the manuscript, provided funding for the study, and critically reviewed and revised the manuscript. A.E. participated in the study design, monitored the recruitment of study subjects, reviewed the medical charts of the cases, took part in the data management, performed data analyses, and critically reviewed and revised the manuscript. P.N. and M.R.R. conceptualized the study, participated in the study design, and contributed to the development of the algorithm for the classification of aetiology and critically reviewed and revised the manuscript. A.M., V.P., and J.G. participated in the study design and contributed to the development of the algorithm for classification of aetiology and critically reviewed and revised the manuscript. M.W. took part in the study design and performed/supervised the MxA analyses and critically reviewed and revised the manuscript. M.L. and M.A. took part in the study design, as well as performed/supervised the PCR analyses and critically reviewed and revised the manuscript. J.G. contributed with input on the study from a diagnostics and global public health perspective and critically reviewed and revised the manuscript. T.A. conceptualized the study, had a leading role in the study design, monitored the study recruitment, provided funding for the study, and critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.05.008.

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