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Improved Diagnostics Help to Identify Clinical Features and Biomarkers That Predict *Mycoplasma pneumoniae* Community-acquired Pneumonia in Children

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Background. There are no reliable signs or symptoms that differentiate *Mycoplasma pneumoniae* (*Mp*) infection in community-acquired pneumonia (CAP) from other etiologies. Additionally, current diagnostic tests do not reliably distinguish between *Mp* infection and carriage. We previously determined that the measurement of *Mp*-specific immunoglobulin M antibody-secreting cells (ASCs) by enzyme-linked immunospot assay allowed for differentiation between infection and carriage. Using this new diagnostic test, we aimed to identify clinical and laboratory features associated with *Mp* infection.

Methods. This is a prospective cohort study of children, 3–18 years of age, with CAP from 2016 to 2017. Clinical features and biomarkers were compared between *Mp*-positive and -negative groups by Mann-Whitney *U* test or Fisher exact test, as appropriate. Area under the receiver operating characteristic curve (AUC) differences and optimal thresholds were determined by using the DeLong test and Youden J statistic, respectively.

Results. Of 63 CAP patients, 29 were *Mp*-positive (46%). *Mp* positivity was statistically associated with older age (median, 8.6 vs 4.7 years), no underlying disease, family with respiratory symptoms, prior antibiotic treatment, prolonged prodromal respiratory symptoms and fever, and extrapulmonary (skin) manifestations. Lower levels of C-reactive protein, white blood cell count, absolute neutrophil count, and procalcitonin (PCT), specifically PCT <0.25 μg/L, were statistically associated with *Mp* infection. A combination of age >5 years (AUC = 0.77), prodromal fever and respiratory symptoms >6 days (AUC = 0.79), and PCT <0.25 μg/L (AUC = 0.81) improved diagnostic performance (AUC = 0.90) (P = .05).

Conclusions. A combination of clinical features and biomarkers may aid physicians in identifying patients at high risk for *Mp* CAP.

Keywords. antibiotics; C-reactive protein; diagnosis; procalcitonin; treatment.

Mycoplasma pneumoniae (*Mp*) is a common bacterial cause of community-acquired pneumonia (CAP) in children [1, 2]. There are substantial challenges in differentiating infection from carriage for *Mp* [3, 4]. Current diagnostic tests, including polymerase chain reaction (PCR) of upper respiratory tract (URT) specimens or serology, do not reliably differentiate between *Mp* infection and carriage [4]. Therefore it is not surprising that previous studies found no signs or symptoms to differentiate *Mp* infection in CAP from other etiology [5, 6], potentially because *Mp* detection was misclassified as infection when in fact it was carriage. *Mp* carriage is estimated to occur in up to 56% of healthy children [4, 5]. *Mp* infection is generally mild and self-limiting, and patients with *Mp* CAP are mostly managed in primary care [7]. The lack of a cell wall makes *Mp* naturally resistant to first-line empirical β-lactam antibiotics for CAP [5]. Empirical macrolide treatment is extensively used to cover potential *Mp* infection, which has led to the emergence of macrolide-resistant *Mp* and a parallel rise in macrolide resistance in other respiratory pathogens [8]. Therefore, with the challenges in diagnostic testing for *Mp* and its limited use in the primary care setting, it is essential to determine clinical features and biomarkers to aid in the diagnosis of *Mp* infection in children with CAP.

We recently demonstrated in a prospective cohort study of CAP in children that the measurement of specific peripheral

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blood immunoglobulin M (IgM) antibody-secreting cells (ASCs) by enzyme-linked immunospot (ELISpot) assay improves diagnosis of *Mp* infection in CAP [9]. This test differentiated between *Mp* infection and carriage. Using this dataset, we here aimed to identify clinical features and biomarkers associated with *Mp* CAP, in which infection may be more accurately identified with the measurement of *Mp*-specific IgM ASCs.

METHODS

Patients

Patients were enrolled between 1 May 2016 and 30 April 2017, at University Children's Hospital Zurich [9]. CAP was clinically defined as the presence of fever >38.5°C and tachypnea according to the British Thoracic Society (BTS) guidelines [2]. Eligible participants were inpatients or outpatients aged 3–18 years. CAP patients <3 years of age were excluded to reduce the probability of viral infection, as it is highest in this age group [10–13]. Patients with hospital-acquired pneumonia, cystic fibrosis, or other chronic lung disorders (excluding asthma), or known primary or secondary immunodeficiencies, were excluded. The local ethics committee approved the protocol for this study (number 2016-00148). Written informed consent was obtained from all parents and children ≥14 years of age.

Specimen Collection and Diagnostic Testing

All enrolled patients were investigated for *Mp* in pharyngeal swab samples by specific real-time PCR [9, 14]. If additional consent was given, blood samples were collected for separation of peripheral blood mononuclear cells (PBMCs) and serum [9]. Serum was stored at −80°C. As detailed elsewhere [9], only CAP patients were included in this study if fresh (isolated ≤4 hours) PBMCs were available to avoid poor ELISpot assay performance, and tested for the presence of *Mp* IgM ASCs. A CAP patient with a positive *Mp* IgM ASC ELISpot assay result was considered to have CAP caused by *Mp* (*Mp* positive). If *Mp* IgM ASCs were not detected, the patient was considered to have CAP caused by another etiology (*Mp* negative). We additionally investigated the frequency of *Mp* by PCR from pharyngeal swabs among household contacts available for sampling at presentation of index patients.

After study closure, pharyngeal swab samples kept at −80°C were additionally tested for *Streptococcus pneumoniae* (*Sp*) by real-time PCR [15], knowing that detection of *Sp* in the URT is likely colonization and not infection [16]. In fact, coinfection with *Sp* and *Mp* is uncommon, whereas co-colonization may be more common [4, 12]. Another 23 viral and bacterial respiratory pathogens were tested using the ePlex respiratory pathogen panel (GenMark Diagnostics, Carlsbad, California), as previously described [17]. In addition, *Mp* serology was performed (Virion\Serion, Würzburg, Germany). The study test results were not available to treating clinicians.

Clinical Data

Demographic, epidemiological, and clinical data were systematically collected using a standardized questionnaire. Full recovery was assessed until 6 months after enrollment. Chest radiographs were ordered for clinical reasons and therefore were not available in 3 (5%) patients. Chest radiographs were assessed by a radiologist during routine clinical care. These radiological findings and the corresponding images were retrospectively reviewed by 2 of the authors, who were blinded to clinical information, using criteria for radiographic pneumonia [18, 19].

Laboratory Data

Blood cell count and C-reactive protein (CRP) analysis was performed as part of routine clinical care. Procalcitonin (PCT) testing was performed retrospectively in a batched analysis and results were not available to treating physicians. PCT levels were measured using a sensitive assay with a detection limit of 0.007 ng/mL (B·R·A·H·M·S PCT sensitive KRYPTOR, ThermoScientific, Berlin, Germany) [20].

Statistical Analysis

The nonparametric Mann-Whitney *U* test was used to compare continuous variables and the Fisher exact test to compare proportions between *Mp*-positive and *Mp*-negative groups. We calculated the area under the receiver operating characteristic (ROC) curve (AUC) of clinical features and biomarkers in differentiating between groups determined by *Mp* IgM ASC ELISpot assay (reference standard). We considered AUC ≥ 0.75 as adequate discrimination [21]. AUC differences were calculated using the DeLong test. Missing values were removed (ignored by roc.formula [22]). The best biomarker threshold was defined as the optimal cutoff that maximized the distance to the identity (diagonal) line in the ROC curve according to Youden J statistic. All reported *P* values are 2-tailed with statistical significance defined as *P* value <.05. Data were analyzed using the R software environment, version 3.6.0 [22].

RESULTS

Study Population

During the 12-month study period, 152 CAP patients were enrolled and *Mp* DNA was detected by PCR in 44 (29%) participants. Of the enrolled population, 63 (41%) CAP patients met the criteria of having fresh PBMCs available to undergo diagnostic testing with the *Mp* IgM ASC ELISpot assay and were included in this study. Of these, 29 (46%) were *Mp*-positive, determined by detection of *Mp* IgM ASCs. As detailed elsewhere [9], all 29 *Mp* IgM ASC-positive patients were also *Mp* PCR positive and IgM positive. However, *Mp* PCR was also positive in 3 (5%) patients who were *Mp* IgM ASC and IgM negative, and *Mp* IgM was found in another 3 (5%) patients who were *Mp* IgM ASC and PCR negative. Chest radiographs were performed in 60 of 63 (95%) included CAP patients, whereof 59 (98%) met the criteria for radiographic pneumonia.

Table 1. Demographic, Epidemiological, and Clinical Characteristics of *Mycoplasma pneumoniae*–Positive Community-acquired Pneumonia (CAP) Versus *M. pneumoniae*–Negative CAP in Children

Characteristics	*Mp*-Positive CAP[a] (n = 29)	*Mp*-Negative CAP[a] (n = 34)	OR (95% CI)	*P* Value
Demographic characteristics				
Age, y[b], median (IQR)	8.6 (6.3–11.0)	4.7 (3.9–6.2)	…	**< .01**
Sex, male	16 (55)	23 (68)	0.6 (.2–1.9)	.44
Season at enrollment				
Spring (March–May)	5 (17)	6 (18)	1.0 (.2–4.4)	1.00
Summer (June–August)	9 (31)	4 (12)	3.3 (.8–16.8)	.07
Autumn (September–November)	9 (31)	8 (23)	1.5 (.4–5.2)	.58
Winter (December–February)	6 (21)	16 (47)	0.3 (.1–1.0)	**.04**
Daycare or preschool attendance	29 (100)	34 (100)	NA	1.00
Immunizations[c]	21/23 (91)	31/32 (97)	0.3 (.0–7.0)	.57
Underlying disease[d]	1 (3)	9 (26)	0.1 (.0–.8)	**.02**
Asthma or history of wheezing	1	1	…	
Cardiovascular	0	0	…	
Gastrointestinal	0	2	…	
Neurological	0	2	…	
Other	0	4	…	
Family with RTI	17 (59)	10 (29)	3.3 (1.1–11.0)	**.02**
Mothers	14	4	…	
Fathers	6	1	…	
Siblings	17	7	…	
Family members with *Mp* detection in URT	17/47 (36)	2/37 (5)	9.7 (2.0–93.2)	**< .01**
Mothers	7/20 (35)	2/26 (8)	6.2 (1.0–69.7)	**.03**
Fathers	1/13 (8)	0/10 (0)	NA	1.00
Siblings	9/14 (64)	0/1 (0)	NA	.40
Symptomatic (RTI)	10/17 (59)	1/2 (50)	1.4 (.0–123.1)	1.00
Asymptomatic (carrier)	7/17 (41)[e]	1/2 (50)[e]	0.7 (.0–62.7)	1.00
Prior antibiotic treatment	13 (45)	5 (15)	4.6 (1.3–19.6)	**.01**
Clinical presentation				
Prodrome				
RTI symptoms, d, median (IQR)	9.0 (6.0–10.0)	4.0 (3.0–7.0)	…	**< .01**
Fever, d, median (IQR)	8.0 (6.0–10.0)	4.0 (3.3–6.0)	…	**< .01**
Fever >2 d[f]	26 (90)	28 (82)	1.8 (.3–12.5)	.49
Fever, °C, at presentation, median (IQR)	39.1 (39.0–39.7)	39.2 (39.0–39.5)	…	.89
RTI symptoms and signs at presentation[g]				
Runny nose	7 (24)	14 (41)	0.5 (.1–1.5)	.19
Sore throat	4 (14)	4 (12)	1.2 (.2–7.1)	1.00
Cough	27 (93)	30 (88)	1.8 (.2–21.2)	.68
Chest pain	3 (10)	7 (21)	0.5 (.1–2.2)	.32
Wheezing	0 (0)	1 (3)	NA	1.00
Abnormal auscultatory findings	19 (66)	24 (71)	0.8 (.2–2.6)	.79
Oxygen saturation < 93%	5 (17)	8 (24)	0.7 (.2–2.8)	.76
Radiographic findings				
Pulmonary infiltrate in chest radiograph[h]	28/28 (100)	31/32 (97)	NA	1.00
Consolidation	18 (64)	25 (78)	0.5 (.1–1.8)	.26
Single lobar infiltrate	13	16	…	
Multilobar infiltrates	5	9	…	
Multilobar infiltrates (unilateral)	1	3	…	
Multilobar infiltrates (bilateral)	4	6	…	
Interstitial	10 (36)	6 (19)	2.4 (.6–9.5)	.16
Reticular	10	6	…	
Nodular	0	0	…	
Pleural effusion	4 (14)	7 (22)	0.6 (.1–2.7)	.52
Severity of illness				
Hospitalization	10 (34)	19 (56)	0.4 (.1–1.3)	.13
LOS, d, median (IQR)	4.5 (3.3–7.0)	2.5 (2.0–5.8)	…	.31
ICU admission	0 (0)	1 (3)	NA	1.00

Table 1. Continued

Characteristics	*Mp*-Positive CAP[a] (n = 29)	*Mp*-Negative CAP[a] (n = 34)	OR (95% CI)	*P* Value
Extrapulmonary manifestation	9 (31)[i]	0 (0)	NA	**< .01**
Dermatological	8	…	…	
Neurological	1	…	…	
Treatment				
Antibiotics after enrollment	27 (93)[j]	31 (91)[j]	1.3 (.1–16.7)	1.00
Amoxicillin ± clavulanic acid	13 (45)	29 (85)	0.1 (.0–.5)	**< .01**
Clarithromycin	10 (34)	1 (3)	16.7 (2.1–772.7)	**< .01**
Doxycycline	10 (34)	1 (3)	16.7 (2.1–772.7)	**< .01**
Other	1 (3)	2 (6)	0.6 (.0–11.6)	1.00
Outcome				
Full recovery	26 (90)[k]	34 (100)	NA	.09
Respiratory sequelae	2	…	…	
Dermatological sequelae	1	…	…	

Data are presented as no. or no. (%) unless otherwise indicated. Differences between groups were determined by the Mann-Whitney *U* test (medians) and Fisher exact test (proportions). *P* values <.05 are indicated in bold.

Abbreviations: CAP, community-acquired pneumonia; CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; LOS, length of hospital stay; *Mp*, *Mycoplasma pneumoniae*; NA, not available; OR, odds ratio; RTI, respiratory tract infection; URT, upper respiratory tract.

[a]Defined according to the *Mp*-specific immunoglobulin M (IgM) antibody-secreting cell (ASC) enzyme-linked immunospot assay results [9].
[b]Only patients between 3 and 18 years of age were enrolled (inclusion criteria) [9].
[c]Per the national immunization schedule in Switzerland.
[d]Chronic lung disorders (excluding asthma) were part of the exclusion criteria [9].
[e]Asymptomatic carriers: *Mp*-positive CAP family members: 6 siblings, 1 mother; *Mp*-negative CAP family members: 1 mother.
[f]According to the prediction rule for risk of *Mp* infection in children with CAP by Fischer et al [23].
[g]Inclusion criteria were clinical diagnosis of CAP with fever >38.5°C and tachypnea according to the British Thoracic Society guidelines [2].
[h]Radiographic evidence of pneumonia was not part of inclusion criteria, but routinely performed in 60 of 63 (95%) included CAP patients; 98% (59/60) met the criteria for radiological pneumonia [18, 19].
[i]Dermatological (n = 8) [32]: *Mp*-induced rash and mucositis (n = 3), urticaria (n = 2), and maculopapular skin eruptions (n = 3); neurological (n = 1): aseptic meningitis.
[j]Antibiotic treatment with ≥1 agent: *Mp*-positive CAP: amoxicillin with or without clavulanic acid + clarithromycin (n = 4), amoxicillin with or without clavulanic acid + doxycycline (n = 3); *Mp*-negative CAP: amoxicillin + clarithromycin (n = 1), amoxicillin with or without clavulanic acid + ceftazidime (n = 1). *Mp*-positive CAP not treated with an antibiotic in vitro active against *Mp*: 9 (31%); all of them fully recovered.
[k]Abnormal outcomes: bronchiolitis obliterans with decreased lung function (n = 1), exertional dyspnea without physical findings, ie, normal lung and cardiac function (n = 1), postinflammatory pigmentary alteration (n = 1).

Clinical Characteristics

The median age of *Mp*-positive patients (8.6 [interquartile range {IQR}, 6.3–11.0] years) was higher compared to *Mp*-negative patients with CAP (4.7 [IQR, 3.9–6.2] years) ($P < .01$; Table 1). *Mp*-positive CAP was more likely than *Mp*-negative CAP to present during summer (odds ratio [OR], 3.3 [95% confidence interval {CI}, .8–16.8]). Underlying disease was less common among *Mp*-positive than *Mp*-negative patients (3% vs 26%; $P = .02$). *Mp*-positive patients were significantly more likely than *Mp*-negative patients to have received prior antibiotic treatment (OR, 4.6 [95% CI, 1.3–19.6]), whereas amoxicillin with or without clavulanic acid was the most frequent agent in both groups (*Mp*-positive, n = 9/13 [69%] vs *Mp*-negative, n = 4/5 [80%]). A positive family history for respiratory tract infection (RTI) was reported in 17 (59%) *Mp*-positive compared to 10 (29%) *Mp*-negative patients (OR, 3.3 [95% CI, 1.1–11.0]; $P = .02$). Household contacts of *Mp*-positive index patients were significantly more likely to have *Mp* detected in the URT than those of *Mp*-negative index patients (OR, 9.7 [95% CI, 2.0–93.2]; $P < .01$). Only 2 family members (5%, both mothers) of *Mp*-negative index patients were PCR positive, of which 1 had RTI symptoms. Among household contacts of *Mp*-positive index patients, *Mp* was detected in the URT of 9 siblings (64%), 7 mothers (35%), and 1 father (8%). Most of them reported having RTI symptoms (59%), but 6 siblings and 1 mother were asymptomatic carriers.

The duration of RTI symptoms and fever prior to CAP diagnosis was longer in *Mp*-positive (median, 9.0 [IQR, 6.0–10.0] days) than *Mp*-negative patients (4.0 [IQR, 3.0–7.0] days; $P < .01$). No other symptoms and signs were statistically different between groups. *Mp*-positive patients were no different than *Mp*-negative patients to have consolidation, interstitial infiltrates, or pleural effusion. Extrapulmonary manifestations were only observed in *Mp*-positive children with CAP (31% vs 0%; $P < .01$) and included dermatological and neurological disorders (Table 1). After inclusion, 9 (31%) of *Mp*-positive patients were not treated with an antibiotic in vitro active against *Mp*.

Hospitalization rates and length of hospital stay were similar between the 2 groups. Detailed characteristics for hospitalized and ambulatory *Mp*-positive and *Mp*-negative patients are shown in Supplementary Tables 1 and 2. Among hospitalized patients, *Mp*-positive patients were more likely to have oxygen demand, extrapulmonary manifestations, and poor outcome than *Mp*-negative patients. Sequelae were only observed

in 3 (10%) hospitalized *Mp*-positive patients (ie, bronchiolitis obliterans, exertional dyspnea, and postinflammatory pigmentary alteration). All *Mp*-negative patients fully recovered.

Laboratory Findings

Hematological assessment together with PCT and CRP analysis for *Mp*-positive and *Mp*-negative CAP patients is shown in Table 2. *Mp*-positive CAP patients had lower white blood cell (WBC) count, absolute neutrophil count (ANC), PCT, and CRP values than *Mp*-negative patients (Figure 1).

Detection of Pathogens

Differences in clinical features and biomarkers may be attributed to another pathogen causing a subset of infections. Therefore, pharyngeal swab samples were tested in this study for other pathogens. Two or more pathogens were found in the URT of 20 (69%) *Mp*-positive and 13 (38%) *Mp*-negative patients (Table 3). *Sp* was equally detected in both cohorts in >60%. Exclusively found in *Mp*-negative patients was respiratory syncytial virus (RSV; n = 8 [24%]; $P < .01$), influenza virus (n = 2), parainfluenza virus (n = 2), and coronavirus (n = 1). No virus was statistically associated with *Mp* positivity. Rhinovirus, adenovirus, and bocavirus were equally detected in both cohorts. Thus, we also compared clinical features and biomarkers of *Mp*-positive patients against the following subgroups of *Mp*-negative patients (n = 34): first, positive for RSV (n = 8, as it is the only virus for which detection in the URT has a high predictive value for CAP etiology [16]); second, positive for other

Table 2. Laboratory Findings of *Mycoplasma pneumoniae*–Positive Community-acquired Pneumonia (CAP) Versus *M. pneumoniae*–Negative CAP in Children

Laboratory Finding	*Mp*-Positive CAP (n = 29)	*Mp*-Negative CAP (n = 34)	OR (95% CI)	*P* Value
Blood cell count				
WBC count, $\times 10^9$ cells/L, median (IQR)	8.97 (6.91–11.80)	12.30 (9.11–20.48)	…	**.01**
Abnormal[a]	5/21 (24)	13/26 (50)	0.3 (.1–1.3)	.08
Leukocytosis	4	12	…	
Leukopenia	1	1	…	
ANC, $\times 10^9$ cells/L, median (IQR)	6.63 (4.21–8.70)	10.00 (6.17–17.29)	…	**.02**
Abnormal[a]	7/21 (33)	14/25 (56)	0.4 (.1–1.5)	.15
Neutrophilia	7	14	…	
Neutropenia	0	0	…	
Lymphocyte count, $\times 10^9$ cells/L, median (IQR)	1.45 (1.16–2.29)	1.77 (1.19–2.58)	…	.52
Abnormal[a]	8/21 (38)	7/24 (29)	1.5 (.4–6.2)	.55
Lymphocytosis	0	0	…	
Lymphopenia	8	7	…	
Monocyte count, $\times 10^9$ cells/L, median (IQR)	0.71 (0.47–0.96)	0.89 (0.63–1.11)	…	.21
Abnormal[a]	2/21 (10)	1/24 (4)	2.4 (.1–148.8)	.59
Monocytosis	2	1	…	
Absolute monocytopenia	0	0	…	
Eosinophil count, $\times 10^9$ cells/L, median (IQR)	0.13 (0.04–0.22)	0.01 (0.00–0.13)	…	.05
Abnormal[a]	3/20 (15)	10/24 (42)	0.3 (.0–1.3)	.09
Eosinophilia	1	2	…	
Absolute eosinopenia	2	8	…	
Basophil count, $\times 10^9$ cells/L, median (IQR)	0.05 (0.02–0.07)	0.04 (0.02–0.07)	…	.98
Abnormal[a]	0/20 (0)	0/24 (0)	NA	1.00
Anemia[a]	2/21 (10)	8/26 (31)	0.2 (.0–1.5)	.15
Platelet count, $\times 10^9$ cells/L, median (IQR)	315 (289–378)	310 (252–356)	…	.27
Abnormal[a]	6/21 (29)	4/25 (16)	2.1 (.4–11.8)	.48
Thrombocytopenia	5	3	…	
Thrombocytosis	1	1	…	
Chemistry				
PCT, µg/L, median (IQR)	0.06 (0.04–0.14)	0.28 (0.12–1.75)	…	**< .01**
CRP, mg/L, median (IQR)	16 (8–36)	72 (24–170)	…	**< .01**
CRP/PCT ratio, mg/µg, median (IQR)	200 (84–452)	104 (39–320)	…	.27
CRP/PCT ratio >400 mg/µg[b]	9 (31)	7 (21)	1.7 (.5–6.5)	.39

Data are presented as no. or no. (%) unless otherwise indicated. Differences between groups were determined by the Mann-Whitney *U* test (medians) and Fisher exact test (proportions). *P* values <.05 are indicated in bold.

Abbreviations: ANC, absolute neutrophil count; CAP, community-acquired pneumonia; CI, confidence interval; CRP, C-reactive protein; IQR, interquartile range; *Mp*, *Mycoplasma pneumoniae*; NA, not available; OR, odds ratio; PCT, procalcitonin; WBC, white blood cell.

[a]Age-specific reference values for hematology were defined as previously described [46].

[b]According to the admission CRP/PCT ratio for risk of *Mp* infection in hospitalized adults with CAP by Neeser et al [24].

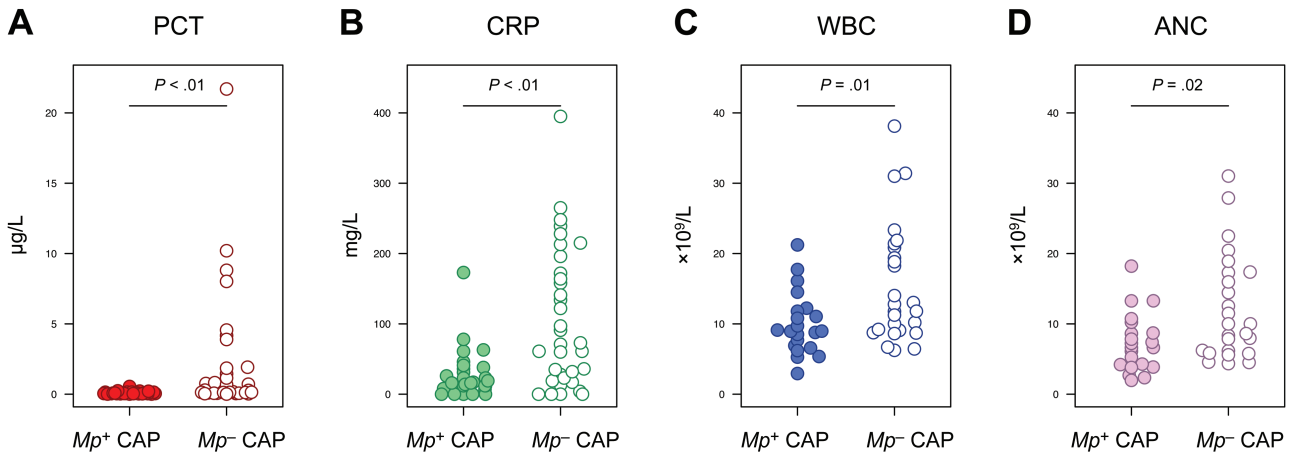

Figure 1. Biomarker test results of *Mycoplasma pneumoniae*–positive community-acquired pneumonia (CAP) vs *M. pneumoniae*–negative CAP in children: procalcitonin (*A*), C-reactive protein (*B*), white blood cell count (*C*), absolute neutrophil count (*D*). The *P* value is indicated in the graphs (Mann-Whitney *U* test). Abbreviations: ANC, absolute neutrophil count; CAP, community-acquired pneumonia; CRP, C-reactive protein; *Mp*, *Mycoplasma pneumoniae*; PCT, procalcitonin; WBC, white blood cell.

viruses than RSV (n = 7, being aware that URT detection of other pathogens than RSV may not be related to pneumonia [3]); and third, negative for viruses (n = 19, in which *Sp* was detected in 14 [74%] in the URT and additionally in 2 of 2 in pleural aspirate during routine clinical care). However, although numbers were again smaller for this subanalysis, *Mp*-positive CAP was also compared to subgroups of *Mp*-negative CAP statistically associated with older age, prolonged prodromal respiratory symptoms, and low levels of PCT (Supplementary Figure 1 and Supplementary Table 3).

ROC Analysis and Performance Curves

ROC analysis was performed for clinical features and biomarkers that were associated with *Mp*-positive CAP. The ROC curves showed good discriminative ability (AUC ≥ 0.75) in differentiating groups for age (AUC = 0.77), duration of prodromal symptoms (AUC = 0.79), PCT (AUC = 0.81), and CRP (AUC = 0.76) (Figure 2A and 2B).

The best clinical threshold to discriminate *Mp*-positive from *Mp*-negative patients was age 5 years and prodromal fever and RTI symptom duration of 6 days (Table 4). The optimal biomarker thresholds were 0.25 µg/L for PCT, 50 mg/L for CRP, 18 × 10^9/L for WBC count, and 8 × 10^9/L for ANC. For assessment of *Mp*-positive CAP, the positive likelihood ratios were highest for age >5 years (2.2 [95% CI, 1.4–3.3]), duration of fever >6 days (3.1 [95% CI, 1.6–5.9]), and RTI symptoms >6 days (2.5 [95% CI, 1.4–4.3]), as well as for PCT <0.25 µg/L (2.2 [95% CI, 1.5–3.2]) and CRP <50 mg/L (2.3 [95% CI, 1.4–3.5]) (Table 4). Using a PCT cutoff of <0.25 µg/L, only 1 (3%) *Mp*-positive patient had a PCT concentration above this cutoff compared with 19 (56%) *Mp*-negative patients (*P* < .01). The *Mp*-positive CAP patient with the high PCT level (ie, 0.55 µg/L) developed bronchiolitis obliterans and dermatological manifestations.

The combination of clinical features and biomarkers was more effective in the diagnosis of *Mp* CAP compared with either clinical features or biomarkers alone (Figure 2C). Improved diagnostic performance was reached by combining clinical features (ie, age with duration of prodromal symptoms: AUC = 0.82 [95% CI, .7–.9]) together with PCT (AUC = 0.90 [95% CI, .8–1.0]) (*P* = .05; Table 4).

DISCUSSION

We evaluated clinical features and biomarkers to diagnose *Mp* infection within a well-defined cohort of ambulatory and hospitalized children with CAP, in which *Mp* infection was diagnosed with the *Mp* IgM ASC ELISpot assay that allows for the differentiation between *Mp* infection and carriage [9]. Diagnosing *Mp* as the cause of CAP at an early stage is important to avoid ineffective first-line empirical β-lactam antibiotics and to evaluate targeted treatment against *Mp* in severe cases [2].

Several scores, ratios, algorithms, and prediction rules have been reported to diagnose *Mp* infection on the basis of clinical features [5, 7, 23, 24]. However, previous studies found no reliable signs or symptoms to differentiate *Mp* infection in CAP from other etiology [5, 6], potentially because *Mp* infection was misclassified as infection when it was carriage. We recently demonstrated that the *Mp* IgM ASC ELISpot assay differentiates *Mp* infection from carriage [9]: While *Mp* DNA and/or IgM were also detected in 48% and 29% healthy control children (n = 21), all were tested negative by the *Mp* IgM ASC ELISpot assay [9]. Notably, the high *Mp* detection rate in CAP patients (46%) in this study may be related to the inclusion age of 3–18 years, in which *Mp* is most frequently detected [1, 2], and the coinciding *Mp* epidemic in Europe during the study period [25–28]. Prevalence estimates are important for translation of diagnostic study findings into clinical practice.

Table 3. Pathogen Detection in Upper Respiratory Tract of *Mycoplasma pneumoniae*–Positive Community-acquired Pneumonia (CAP) Versus *M. pneumoniae*–Negative CAP in Children

Pathogen	*Mp*-Positive CAP (n = 29)	*Mp*-Negative CAP (n = 34)	OR (95% CI)	*P* Value
Pathogen				
Any pathogen	29 (100)	29 (85)	NA	.06
≥2 pathogens	20 (69)	13 (38)	3.5 (1.1–11.7)	**.02**
≥3 pathogens	7 (24)	5 (15)	1.8 (.4–8.4)	.52
Bacteria				
Any bacteria	29 (100)	26 (76)	NA	**< .01**
Mp	29 (100)	3 (9)	NA	**< .01**
Sp	18 (62)	26 (76)[a]	0.5 (.1–1.7)	.27
Chlamydophila pneumoniae	0 (0)	1 (3)	NA	1.00
Legionella pneumophila	0 (0)	0 (0)	NA	1.00
Bordetella pertussis	0 (0)	0 (0)	NA	1.00
Viruses				
Any virus	9 (32)	15 (45)	0.6 (.2–1.8)	.31
RSV	0 (0)	8 (24)[b]	NA	**< .01**
A	…	2	…	
B	…	3	…	
Influenza virus	0 (0)	2 (6)	NA	.50
A	…	2	…	
A(H1)	…	0	…	
A(2009 H1N1)	…	0	…	
A(H3)	…	1	…	
B	…	0	…	
Parainfluenza virus	0 (0)	2 (6)	NA	.50
1	…	0	…	
2	…	2	…	
3	…	0	…	
4	…	0	…	
Human rhinovirus/enterovirus[c]	3 (11)	4 (12)	0.9 (.1–5.7)	1.00
Adenovirus	5 (18)	3 (9)	2.1 (.4–15.3)	.45
Human metapneumovirus	0 (0)	0 (0)	NA	1.00
Coronavirus	0 (0)	1 (3)	NA	1.00
229E	…	0	…	
HKU1	…	0	…	
NL63	…	0	…	
OC43	…	1	…	
MERS coronavirus	…	0	…	
Human bocavirus	1 (4)	1 (3)	1.2 (.0–95.8)	1.00

Data are presented as no. or no. (%). Differences between proportions were determined by the Fisher exact test. *P* values <.05 are indicated in bold. Pharyngeal samples were tested in all patients by real-time polymerase chain reaction (PCR) for *Mp* [14] and *Sp* [15], and for other bacterial and viral pathogens by the ePlex respiratory panel [17] in 28 of 29 *Mp*-positive CAP and 33 of 34 *Mp*-negative CAP patients with residual respiratory samples. Blood cultures were performed in 14 (48%) *Mp*-positive CAP and 20 (59%) *Mp*-negative CAP patients during routine clinical care and all were negative. Routine PCR testing for *Mp* [14] as part of clinical care was positive in 8 of 8 *Mp*-positive CAP and 0 of 11 *Mp*-negative CAP patients.

Abbreviations: CAP, community-acquired pneumonia; CI, confidence interval; MERS, Middle East respiratory syndrome; *Mp, Mycoplasma pneumoniae*; NA, not available; OR, odds ratio; RSV, respiratory syncytial virus; *Sp, Streptococcus pneumoniae*.

[a]Two patients had *Sp* detected in both upper respiratory tract (URT) and pleural fluid samples (pleural puncture performed during routine clinical care). They had no other pathogens detected in the URT apart from *Sp*.

[b]RSV was additionally detected in nasopharyngeal samples of 3 patients by rapid antigen detection test (Sofia, Quidel, San Diego, California) during routine clinical care.

[c]No differentiation possible between rhinovirus and enterovirus [17].

When taking the Bayes theorem into account, the posttest probability of disease presence will be higher given a higher pretest probability or prevalence [29–31]. For proper implementation of a new diagnostic test into clinical practice, it is therefore necessary that the test will be assessed in the context of all the other diagnostic information that is available at the time point of testing.

We were able to corroborate previous study findings in that *Mp*-positive CAP manifests predominantly in school-aged children >5 years [1, 12, 23] and children present with prodromal fever and respiratory symptoms of >6 days [1], significantly longer compared with CAP caused by other etiology. Other symptoms and signs, as well as radiographic findings, did not differentiate *Mp*-positive from *Mp*-negative CAP [1, 5, 6].

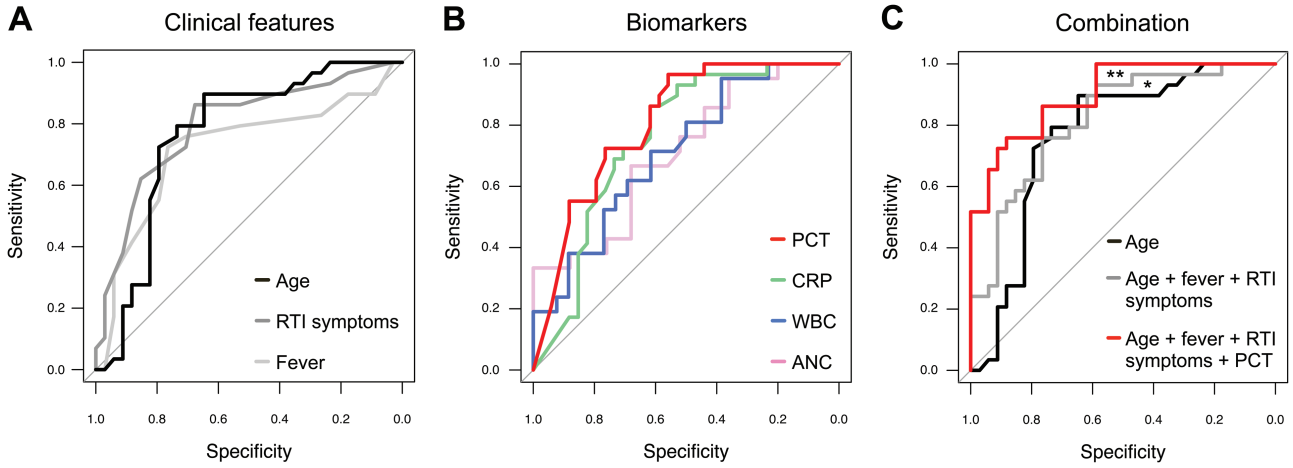

Figure 2. Receiver operating characteristic (ROC) curve of sensitivity vs specificity for clinical features (*A*), biomarkers (*B*), and a combination of both (*C*). Area under the ROC curve (AUC) differences (DeLong test): *A* and *B*, No significant AUC differences. *C*, **P* = .37 (age vs combination of clinical features); ***P* = .05 (combination of clinical features vs combination of clinical features plus procalcitonin). Abbreviations: ANC, absolute neutrophil count; CRP, C-reactive protein; PCT, procalcitonin; RTI, respiratory tract infection; WBC, white blood cell.

Extrapulmonary manifestations, predominantly skin disorders, were statistically associated with *Mp*-positive CAP in this study. Its presence significantly increases the probability of underlying *Mp* infection [32]. This may be also true for the presence of RTI within families of *Mp*-positive CAP patients. Our observation of frequent RTI symptoms and *Mp* detection in members of those families supports previous data about *Mp* spread among persons in close contact and family transmission of *Mp* [33–36].

Mp infection is mild and self-limiting in most cases, and manifests predominantly in previously healthy children [5]. In our study, one-third of *Mp*-positive CAP patients were not treated with an antibiotic in vitro active against *Mp*, but all of these children fully recovered. The BTS guidelines advise that macrolide antibiotics may be added at any age in case of very severe disease or if there is no response to first-line empirical treatment [2]. Prior antibiotic treatment was associated with *Mp* CAP and included treatment with β-lactam antibiotics in most cases. This supports that in patients that do not respond to β-lactam antibiotics, *Mp* CAP should be considered [2, 7]. We did not find statistical differences in fever duration following CAP diagnosis, hospital length of stay, or recovery at follow-up between *Mp*-positive patients who did and did not receive antibiotics against *Mp* (data not shown). These findings support the need for future interventional studies assessing the effect of antibiotics for *Mp* CAP [1, 9, 37, 38].

Our study demonstrated that in *Mp*-positive CAP biomarker levels of PCT, CRP, WBC count, and ANC were statistically lower compared with *Mp*-negative CAP. Despite limited numbers, this

Table 4. Best Thresholds With Diagnostic Accuracy for *Mycoplasma pneumoniae*–Positive Community-acquired Pneumonia (CAP) Versus *M. pneumoniae*–Negative CAP in Children

Parameter	Threshold	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	AUC (95% CI)
Clinical features						
Age, y	>5	0.90 (.74–.96)	0.59 (.42–.74)	2.18 (1.43–3.31)	0.18 (.06–.53)	0.77 (.64–.89)
Fever, d	>6	0.72 (.54–.85)	0.76 (.60–.88)	3.08 (1.61–5.87)	0.36 (.19–.67)	0.72 (.59–.86)
RTI symptoms, d	>6	0.72 (.54–.85)	0.71 (.54–.83)	2.46 (1.40–4.34)	0.39 (.21–.73)	0.79 (.68–.91)
Biomarkers						
PCT, µg/L	<0.25	0.97 (.83–.99)	0.56 (.39–.71)	2.19 (1.49–3.21)	0.06 (.01–.43)	0.81 (.70–.92)
CRP, mg/L	<50	0.86 (.69–.95)	0.62 (.45–.76)	2.25 (1.44–3.54)	0.22 (.09–.58)	0.76 (.63–.88)
WBC count, $\times 10^9$ cells/L	<18.00	0.95 (.77–.99)	0.38 (.22–.57)	1.55 (1.13–2.13)	0.12 (.02–.89)	0.71 (.56–.86)
ANC, $\times 10^9$ cells/L	<8.00	0.67 (.45–.83)	0.60 (.41–.77)	1.67 (.94–2.94)	0.56 (.28–1.10)	0.71 (.56–.86)
Combination						
Age + fever + RTI symptoms	As defined above	1.00 (.88–1.00)	0.44 (.29–.61)	1.79 (1.33–2.41)	NA	0.82 (.71–.92)
Age + fever + RTI symptoms + PCT	As defined above	1.00 (.88–1.00)	0.32 (.19–.49)	1.48 (1.17–1.87)	NA	0.90 (.83–.97)

The threshold is the optimal cutoff that maximizes the distance to the identity (diagonal) line in the receiver operating characteristic curve in Figure 2 according to the Youden J statistic using the "coords" function in R software environment (version 3.6.0) [22].

Abbreviations: ANC, absolute neutrophil count; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CRP, C-reactive protein; LR, likelihood ratio; NA, not available; PCT, procalcitonin; RTI, respiratory tract infection; WBC, white blood cell.

was even true for PCT and CRP of *Mp*-positive CAP in comparison to viral CAP (RSV) as a subgroup of *Mp*-negative CAP. Similar trends have been observed in previous CAP studies for CRP, WBC count, and ANC [23, 39–43], and recently for PCT with median levels from 0.05 to 0.19 µg/L in CAP considered to be caused by *Mp* [39–41, 44]. A study with conflicting results suggesting higher PCT levels associated with *Mp* may be hampered by diagnostics [45]. A PCT cutoff of <0.25 µg/L reached the best discriminatory power in differentiating *Mp*-positive from *Mp*-negative children with CAP, which is supported by previous studies [39–41, 44]. Only 1 *Mp*-positive patient had a PCT level of 0.55 µg/L and was above this cutoff.

Our study has several limitations. First, though sampling was performed in a relevant population of clinical CAP [9], the study population is small and represents a convenience sample from an observational study, and we cannot rule out that unintended selection occurred. However, even though the sample size is small for prediction, the scope of this study was to describe risk factors for *Mp* infection. Second, mild cases may not have been referred to our tertiary center. Third, the study enrolled children from 3 to 18 years of age to reduce the probability of viral infection [9]. However, younger children with *Mp* infection may have, more likely, other RTIs than CAP [43].

In conclusion, improved diagnostics helped to identify clinical features and biomarkers that may predict *Mp* CAP in children, such as age >5 years, no underlying disease, family with RTI, prior antibiotic treatment, prodromal respiratory symptoms and fever >6 days, as well as extrapulmonary (skin) manifestations. Biomarkers showed some differences between *Mp*-positive and *Mp*-negative patients in that *Mp* positivity was associated with low levels of CRP, WBC count, ANC, and PCT, particularly a PCT cutoff of <0.25 µg/L. However, the study size is small and even the best combinations of clinical features and biomarkers may not be reliable enough to be used as a diagnostic itself. Our data support that a combination of clinical features and biomarkers may help physicians in identifying patients at high risk for *Mp* CAP, which warrants further investigations in a larger cohort of children with *Mp* infection.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. P. M. M. S. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study design: P. M. M. S. and approved by all authors. Acquisition of data: P. M. M. S., S. K., M. S., P. P., C. R., C. K., T. H., C. G. Analysis and interpretation of data: P. M. M. S., L. A., L. M. B., A. M. C. v. R., C. B. Drafting of the manuscript: P. M. M. S. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: P. M. M. S., L. A., L. M. B. Obtained funding: P. M. M. S. Administrative, technical, or material support: P. M. M. S., M. S., C. K., T. H., C. G., C. B.

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