

Supplementary Material

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1 SUPPLEMENTARY METHODS

1.1 Eligibility criteria employed in the original studies

CURIOSITY [1]

Inclusion criteria: clinical suspicion of an acute infectious disease, peak fever $>37.5^{\circ}\text{C}$ since onset of symptoms, and duration of symptoms ≤ 12 days.

Exclusion criteria: evidence of acute infection in the two weeks preceding enrollment; congenital immune deficiency; treatment with immunosuppressive or immunomodulatory therapy; active malignancy; and human immunodeficiency virus (HIV), or hepatitis B/C virus infection.

OPPORTUNITY [2]

Inclusion criteria: patients aged between 2 and 60 months presenting with fever (peak temperature $\geq 38.0^{\circ}\text{C}$ measured by axillary, rectal, or ear thermometer) and symptoms of lower respiratory tract infection or fever without source existing for a maximum of 6 days.

Exclusion criteria: previous episode of fever in the past 3 weeks; psychomotor retardation; moderate to severe metabolic disorder; primary or secondary immunodeficiency; HIV, infection by hepatitis B or hepatitis C viruses; and active malignancies.

1.2 Laboratory procedures

In the CURIOSITY study, nasal swabs were stored at 4°C for up to 72h before transport to a central laboratory, where two multiplex PCR analyses were conducted to detect common respiratory viral (Seeplex RV15) and bacterial (Seeplex PB6) pathogens. Venous blood specimens were stored at 4°C for up to 5h before fractionation into serum and plasma and storage at -80°C . Host-protein biomarkers were measured as follows: CRP using either Cobas-6000, Cobas-Integra-400/800, or Modular-Analytics- P800 (Roche); TRAIL and IP-10 using ImmunoXpert™ (MeMed).

In the OPPORTUNITY study, nasal swabs were collected (universal transport medium, Copan, Brescia, Italy), frozen within 2h and stored at -80°C before transport to the MeMed laboratory, where multiplex PCR testing was performed for 15 common respiratory viruses (Seeplex RV15, Seegene, Seoul, South Korea). Similarly, venous blood specimens were fractionated into serum and plasma within 2h of collection and stored at -80°C before transport to the MeMed laboratory; TRAIL and IP-10 measurements were performed using ImmunoXpert™ (MeMed) and CRP was measured using Cobas e501 or Cobas-6000.

1.3 Statistical analysis

Sensitivity was defined as the number of patients that received a bacterial reference standard and a bacterial BV score (score >65), divided by the number of patients that received a bacterial reference standard. Specificity was symmetrically defined as the number of patients that received a viral reference standard and a viral BV score (score <35), divided by the number of patients that received a viral reference standard. The percentage of cases assigned an equivocal score ($35 \leq \text{score} \leq 65$) was reported. Fisher's exact test was used for comparing proportions.

Positive predictive value (PPV) was defined as the number of patients that received a bacterial reference standard and a bacterial index test score (score > 65), divided by the number of patients that received a bacterial index test score. Negative predictive value (NPV) was symmetrically defined as the number of patients that received a viral reference standard and a viral index test score (score < 35), divided by the number of patients that received a viral index test score.

2 SUPPLEMENTARY TABLES

2.1 Supplementary Table 1. Reference standard bacterial cases (n=12)

Patient 627	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	100	Bacterial	F	20	Gastroenteritis	40	2	Ciproxin
	Microbiology			CXR	LAB	CRP	Clinical details	
	Positive Adenovirus. Negative stool cultures.			Normal	WBC 12.5K, ANC 11K	346.9	Fever and vomiting 3 days before admission. Normal physical examination.	
Patient 3887	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	98	Bacterial	F	0.7	Pyelonephritis	40.5	6	Augmentin, Gentamycin, Ceftriaxon, Co-trimoxazol
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive Adenovirus and Bocavirus. Positive E.coli urine culture. Negative blood culture.		NA	WBC 32K, ANC NA	122.2	Fever and vomiting 1 day before admission. In physical examination - ill-appearing, without any other significant sign. In US, unilateral enlarged pyelum, indicative of pyelonephritis.		
Patient 591	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	97	Bacterial	F	16	Acute Tonsillitis	39.2	2	During hospitalization with Penicillin G, Discharged with Moxypen
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive Adenovirus. Negative blood culture. Positive Group G strep nasopharyngeal culture. Positive Mycoplasma pneumoniae (MP) IgM, EBV negative IgM, CMV negative IgM positive IgG, Q-fever negative IgM.		NA	WBC 10K, ANC 7K	135.9	Fever and sore throat 3 weeks before admission. In physical examination enlarged tonsils and enlarged cervical lymph nodes.		
Patient 479	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx

	97	Bacterial	M	3.5	Pneumonia	39	2	During hospitalization with Cefuroxime, Discharged with Moxypen
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive Adenovirus and Parainfluenza virus 4. Positive Streptococcus pneumoniae (SP) and Haemophilus influenza (HI) PCR. Negative Mycoplasma pneumoniae (MP) IgM.		LLL consolidation	WBC 33K, ANC 30K	85.8	Fever, cough and vomiting from day of admission. In physical examination decreased breath sounds at the base left lung.		
Patient 3530	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	89	Bacterial	M	2	Pneumonia	40	4	Treated with Azenil prior to admission, treats with Ampicillin during hospitalization, Discharged with Moxypen
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive Adenovirus. Negative blood culture.		RUL Consolidation	WBC 27K, ANC 18K	105.0	Fever and cough 5 day before admission. In physical examination decreased breath sounds at the right lung.		
Patient 483	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	81	Bacterial	M	2.2	Acute Tonsillitis	40.7	0	Discharged with Moxypen
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive Adenovirus. Positive Streptococcus pneumoniae (SP) and Haemophilus influenza (HI) PCR. Positive Mycoplasma pneumoniae (MP) IgM, EBV CMV negative IgM igG. Negative blood culture.		Normal	WBC 19K, ANC 13K	20.6	Fever 1 day before admission. In physical examination, bilateral TM erythema and enlarged tonsils with exudate.		
Patient 3349	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx

	75	Bacterial	F	3.2	Cellulitis	NA	3	Treates with Moxypen prior to admission, treates with Cefamezin during hospitalization, Discharged with Ceforal
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive Adenovirus. Negative blood and urine cultures.		Normal	WBC 21K, ANC 13K	78.3	Fever and cellulitis 2 days before admission. In physical examination angular erythema above the left ankle and erythematous tonsils.		
Patient 702	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	62	Bacterial	F	5	Kawasaki; Gastroenteritis	40.3	5	NA
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive campylobacter stool culture, positive Adenovirus, negative urine, blood and nasopharyngeal culture, EBV CMV negative IgM igG, positive Haemophilus influenza (HI) PCR		NA	WBC 8K, ANC 5K	44.9	Fever, vomiting and diarrhea 5 days before admission, rash from day of admission. Physical examination: diffuse maculopapular rash, non purulent bilateral conjunctivitis, strawberry tongue and cracked lips. enlarged unilateral lymph node, systolic heart murmur. Treated with IVIG and aspirin for Kawasaki disease.		
Patient 3796	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	58	Bacterial	F	3	AOM	39.01	2	Treates with Augmentin prior to admission, Augmentin treatment was continued.
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive Adenovirus		NA	WBC 13K, ANC 11	173.5	Fever, apathy and otodynia 3 days before admission. In physical examination - ill-appearing, unilateral ear bulging, enlarged tonsils		
Patient 351	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	56	Bacterial	M	1.5	Pneumonia	40	NA	Discharged with Moxypen

	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive Adenovirus, positive GAS nasopharyngeal culture, Negative blood culture. Normal urine stick, positive Streptococcus pneumoniae (SP) and Haemophilus influenza (HI) PCR.		LLL consolidation	WBC 15K, ANC 7K	112.0	Fever 3 days before admission, Known background of minimal hydronephrosis. In physical examination enlarged tonsils with exudate.		
Patient 963	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	53	Bacterial	M	0.5	Gastroenteritis	40	NA	Discharged with Azenil
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive campylobacter stool culture, positive Adenovirus, positive Streptococcus pneumoniae (SP) PCR		NA	WBC 15K, ANC 9K	74.5	Fever and diarrhea 5 days before admission, on day of admission bloody stool. Normal physical examination		
Patient 3745	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	49	Bacterial	M	0.9	Bronchiolitis	38.5	13	Augmentin
	Microbiology		CXR	LAB	CRP	Clinical details		
	Positive RSV, Adenovirus, HMPV, Bocavirus PCR, Positive Adenovirus Ag in stool, negative Noro and Rota virus stool Ag		Bilateral pneumonia and atelectasis	WBC 24K (max 28K), ANC 20K	26.4	Fever, dyspnea, cough and rhinoreah 2 days before admission. Background of prematurity and IRDS. In physical examination - ill-appearing, saturation 85%, rales, crepitation, wheezing, prolonged expiration and accessory muscle use. Was hospitalized in PICU and intubated, lowest saturation 48%.. Complaints and lab resolved without intervention. Follow up - still hospitalized in PICU		

2.2 Supplementary Table 2. Cases where the BV score was bacterial and the reference standard was viral (false positives, n=10)

Patient 3539	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	99	Viral	M	1	Upper respiratory tract infection	39.2	2	Not given
	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus, Enterovirus and Rhinovirus Negative blood culture			LLL consolidation (official result), RML consolidation in hospitalization summary, in revision- no finding.	WBC 7.63K, ANC 0.6K	Fever, cough and rhinorrhea a day before admission. In physical examination, moaning, bronchial breath sounds.		
Patient 1255	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	96	Viral	M	2.5	Acute tonsillitis	39.8	4	Not given
	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus Negative blood culture			Normal	WBC 13K, ANC 10K	Fever and vomiting 4 days before admission. Background of prematurity. Erythema in pharynx in physical examination. Complaints and lab resolved without intervention.		
Patient 1337	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	93	Viral	M	1.5	Gastroenteritis	39.5	3	Ceftriaxone IV
	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus, Coronavirus OC43 and Rhinovirus Negative blood and stool culture.			Not done	WBC 26K, ANC 13K	Fever, vomiting and diarrhea a day before admission. Physical examination normal except for lymphadenopathy. Improvement after treatment.		
Patient 3125	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	92	Viral	F	0.45	Bronchiolitis with secondary bacterial infection	38.5	4	Treated with Moxypen, discharged with Moxypen
	Microbiology			CXR	LAB	Clinical details		

	Positive Adenovirus and parainfluenza, negative Influenza and RSV Negative blood culture			Normal	WBC 13K, ANC 6	Fever and dyspnea 4 days before admission. In physical examination dyspnea and tachypnea, diffuse rales, prolonged expiration, decreased breathing sounds.		
Patient 1360	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	89	Viral	M	4.5	Acute tonsillitis	39.1	4	Ampicillin IV was started and stopped after appearance of rash.
	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus Negative nasopharyngeal, blood and urine culture, EBV, CMV IgG positive, IgM negative			Normal	WBC 5K, ANC 3K	Fever, cough, sore throat and abdominal pain a week before admission. In physical examination enlarged tonsils with exudate, lymphadenopathy, splenomegaly. Abx was started and stopped due to rash which was suspected as viral infection.		
Patient 3955	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	84	Viral	F	2	Upper respiratory tract infection	38.7	2	Not given
	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus, Enterovirus and Rhinovirus			Not done	WBC unknown, ANC unknown	Fever, cough and dyspnea a day before admission. In physical examination accessory muscle use, rales and prolonged expiration.		
Patient 563	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	81	Viral	M	1.5	Acute tonsillitis	41	Unknown	Not given
	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus			Normal	WBC 17K, ANC 9K	Fever, cough, rhinorrhea and decreased appetite 3 days before admission. HRAD background. In physical examination ill appearing, erythema in pharynx and upper respiratory rales. Was hospitalized for observation, improved without treatment.		
Patient 3056	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	78	Viral	M	1.75	Acute tonsillitis	40	0	Not given

	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus and Parainfluenza Negative nasopharyngeal and blood culture culture			Normal	WBC 27K, ANC 8.4K	Fever, dyspnea, dry cough and decreased appetite 3 days before admission. Known background of recurrent tonsilitis and AOM. In physical examination enlarged tonsils with exudate and upper respiratory rates. No findings of abscess in ENT examination.		
Patient 3477	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	75	Viral	F	2	Unspecified viral infection	38.5	2	Not given
	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus and Rhinovirus			Not done	WBC 6.3K	Fever and rhinorrhea 3 days before admission. Vomiting from day of admission. Parent with acute tonsillitis treated with abx. Suspected AOM in physical examination, in second examination no finding.		
Patient 672	BV	Experts	Sex	Age (y)	Discharge diagnosis	Max temp	Hospitalization Duration	Abx
	72	Viral	F	1	Gastroenteritis	38.5	3	Not given
	Microbiology			CXR	LAB	Clinical details		
	Positive Adenovirus, Bocavirus, Rhinovirus, Streptococcus pneumoniae and Haemophilus influenza. Positive Norovirus in stool PCR, positive Clostridium difficile Toxin B, Negative stool and blood culture			Not done	WBC 10K, ANC 5K	Fever, vomiting and diarrhea, 4 days before admission contacted ED, was diagnosed as viral GE, treated with fluids and discharged home. Returned to the ED because of no improvement. known background of congenital hypothyroidism. In physical examination: conjunctivitis. Admitted and treated with fluids, improvement after treatment.		

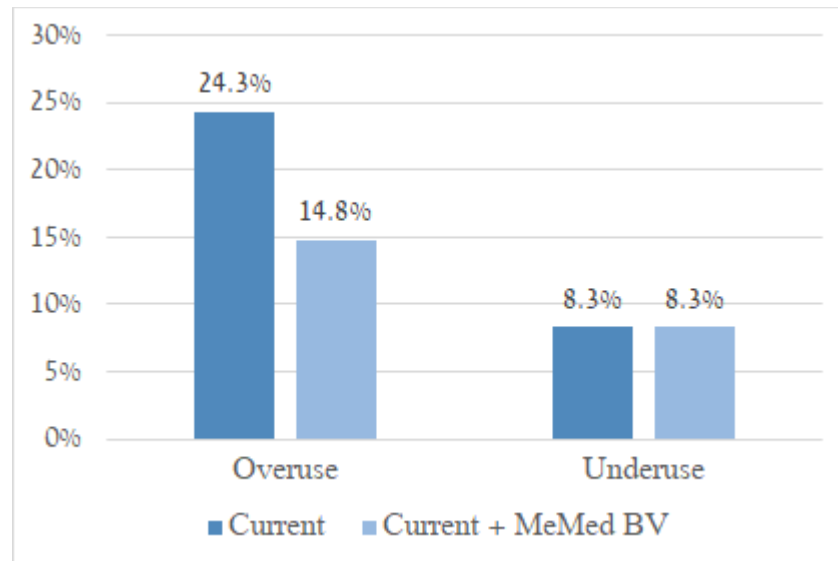
2.3 Supplementary Table 3. BV score comparison to routine biomarkers

	n = 127	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Equivocal rate %
Score	< 35 or > 65	100.0 (100.0- 100.0)	89.5% (83.2-95.8)	41.2 (15.1- 67.3)	100.0 (92.6- 100.0)	19.7
CRP	20	100.0 (100.0- 100.0)	33.0 (24.3- 41.8)	13.5 (6.2- 20.7)	100.0 (52.6- 100.0)	
	40	83.3 (58.6- 100.0)	59.1 (50.0- 68.3)	17.5 (7.4- 27.7)	97.1 (78.9- 100.0)	
	60	75.0 (46.3- 100.0)	74.8 (66.7- 82.8)	23.7 (9.5- 37.8)	96.6 (84.9- 100.0)	
	80	58.3 (25.6- 91.1)	88.7 (82.8- 94.6)	35.0 (12.1- 57.9)	95.3 (88.9- 100.0)	
	< 20 or > 80	100.0 (100.0- 100.0)	74.5 (62.1- 86.9)	35.0 (12.1- 57.9)	100.0 (80.5- 100.0)	54.3
WBC	15,000	66.7 (35.4- 98.0)	64.3 (55.3- 73.3)	16.7 (5.7- 27.6)	94.7 (78.9- 100.0)	
ANC	10,000	55.6 (15.0- 96.1)	74.5 (66.1- 83.0)	15.6 (2.3- 28.9)	95.2 (83.2- 100.)	

CRP, C-reactive protein; WBC, white blood cells; ANC, absolute neutrophil count; PPV, positive predictive value; NPV, negative predictive value.

3 SUPPLEMENTARY FIGURES

3.1 Supplementary Figure 1. Potential impact of BV score on antibiotic overuse and underuse.



The calculations and assumptions are explained in Methods. Data is shown for the analysis cohort; n=127.

4 REFERENCES

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