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°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 ≥38.0°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≤score≤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.
## SUPPLEMENTARY TABLES

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

| Patient | BV | Experts | Sex | Age (y) | Discharge diagnosis | Max temp | Hospitalization Duration | Abx |
|---------|----|---------|-----|---------|---------------------|----------|--------------------------|-----|
| 627     |    |         | F   | 20      | Gastroenteritis     | 40       | 2                        | Ciproxin |
|         |    |         |     |         |                     |          |                          |      |
|         |    |         |     |         | Microbiology        |          |                          |      |
|         |    |         |     |         | CXR                 |          |                          |      |
|         |    |         |     |         | LAB                 |          |                          |      |
|         |    |         |     |         | CRP                 |          |                          |      |
|         |    |         |     |         | Clinical details    |          |                          |      |
|         |    |         |     |         | Positive Adenovirus, Negative stool cultures. |          |                          |      |
| 3887    |    |         | 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. |          |                          |      |
| 591     |    |         | 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. |          |                          |      |

Patient 479

| Patient | BV | Experts | Sex | Age (y) | Discharge diagnosis | Max temp | Hospitalization Duration | Abx |
|---------|----|---------|-----|---------|---------------------|----------|--------------------------|-----|
| 479     |    |         | F   |         |                     |          |                          |     |
|         |    |         |     |         | Microbiology        |          |                          |     |
|         |    |         |     |         | CXR                 |          |                          |     |
|         |    |         |     |         | LAB                 |          |                          |     |
|         |    |         |     |         | CRP                 |          |                          |     |
|         |    |         |     |         | Clinical details    |          |                          |     |
|         |    |         |     |         | Positive Adenovirus. Negative blood culture. In physical examination enlarged tonsils and enlarged cervical lymph nodes. |          |                          |     |
| Patient | BV | Experts | Sex | Age (y) | Discharge diagnosis | Max temp | Hospitalization Duration | Abx |
|---------|----|---------|-----|---------|---------------------|----------|--------------------------|-----|
| 3530    | 89 | Bacterial | M   | 2       | Pneumonia           | 40       | 4                        |     |
|         |     |          |     |         |                     |          |                          |     |
| 483     | 81 | Bacterial | M   | 2.2     | Acute Tonsillitis   | 40.7     | 0                        |     |

**Clinical details for Patient 3530:**
- 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
- Clinical details: Fever, cough and vomiting from day of admission. In physical examination decreased breath sounds at the base left lung.

**Clinical details for Patient 483:**
- Positive Adenovirus. Negative blood culture.
- RUL Consolidation
- WBC 27K, ANC 18K
- Clinical details: Fever and cough 5 day before admission. In physical examination decreased breath sounds at the right lung.

**Clinical details for Patient 3349:**
- 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
- Clinical details: Fever 1 day before admission. In physical examination, bilateral TM erythema and enlarged tonsils with exudate.
| Patient 702 | 75 | Bacterial | F | 3.2 | Cellulitis | NA | 3 | Treates with Moxypen prior to admission, treats 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 3796 | 62 | Bacterial | F | 5 | Kawasaki; Gastroenteritis | 40.3 | 5 | NA |
| BV | Experts | Sex | Age (y) | Discharge diagnosis | Max temp | Hospitalization Duration | Abx |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 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 unilaterial lymph node, systolic heart murmur. Treated with IVIG and aspirin for Kawasaki disease. |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Patient 351 | 58 | Bacterial | F | 3 | AOM | 39.01 | 2 | Treates with Augmentin prior to admission, Augmentin treatment was continued. |
| BV | Experts | Sex | Age (y) | Discharge diagnosis | Max temp | Hospitalization Duration | Abx |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 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 |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 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 |
|--------------|-----|-----|-----|-----------------|
| Positivecampylobacter 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 rhinorrhea 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 | BV | Experts | Sex | Age (y) | Discharge diagnosis | Max temp | Hospitalization Duration | Abx |
|---------|----|---------|-----|---------|---------------------|----------|--------------------------|-----|
| 3539    | 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. |
| 1255    | 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. |
| 1337    | 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. |
| 3125    | 92 | Viral   | F   | 0.45    | Bronchiolitis with secondary bacterial infection | 38.5     | 4                        | Treated with Moxypen, discharged with Moxypen |
| Microbiology | CXR | LAB | Clinical details |
| Patient 1360 | BV | Experts | Sex | Age (y) | Discharge diagnosis | Max temp | Hospitalization Duration | Abx |
|--------------|----|---------|-----|---------|---------------------|----------|--------------------------|-----|
|              |    |         |     |         | Positive Adenovirus and parainfluenza, negative Influenza and RSV | 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. |

**Clinical details**

**Microbiology**

- 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 |
|--------------|----|---------|-----|---------|---------------------|----------|--------------------------|-----|
|              |    |         |     |         | 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. |

**Microbiology**

- 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 563 | BV | Experts | Sex | Age (y) | Discharge diagnosis | Max temp | Hospitalization Duration | Abx |
|--------------|----|---------|-----|---------|---------------------|----------|--------------------------|-----|
|              |    |         |     |         | Positive Adenovirus | 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. |

**Clinical details**

**Microbiology**

- 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 |
|--------------|----|---------|-----|---------|---------------------|----------|--------------------------|-----|
|              |    |         |     |         | Positive Adenovirus | 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. |

**Clinical details**

**Microbiology**

- 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.
| Microbiology | CXR | LAB | Clinical details |
|--------------|-----|-----|-----------------|
| Positive Adenovirus and Parainfluenza Negative nasopharyngeal and blood culture | Normal | WBC 27K, ANC 8.4K | Fever, dyspnea, dry cough and decreased appetite 3 days before admission. Known background of recurrent tonsillitis and AOM. In physical examination enlarged tonsils with exudate and upper respiratory rales. No findings of abscess in ENT examination. |

|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

| 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. |

|BV| Experts| Sex| Age (y)| Discharge diagnosis| Max temp| Hospitalization Duration| Abx|
|---|---|---|---|---|---|---|---|
|72| Viral| F| 1| Gastroenteritis| 38.5| 3| Not given|

Microbiology

| 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. |

|BV| Experts| Sex| Age (y)| Discharge diagnosis| Max temp| Hospitalization Duration| Abx|
|---|---|---|---|---|---|---|---|
|672| Viral| F| 1| | | | |
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 (83.2-100.0) |  |

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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