Detectable A Disintegrin and Metalloproteinase With Thrombospondin Motifs-1 in Serum Is Associated With Adverse Outcome in Pediatric Sepsis

IMPORTANCE: A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 is hypothesized to play a role in the pathogenesis of invasive infection, but studies in sepsis are lacking.

OBJECTIVES: To study A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 protein level in pediatric sepsis and to study the association with outcome.

DESIGN: Data from two prospective cohort studies.

SETTING AND PARTICIPANTS: Cohort 1 is from a single-center study involving children admitted to PICU with meningococcal sepsis (samples obtained at three time points). Cohort 2 includes patients from a multicenter study involving children admitted to the hospital with invasive bacterial infections of differing etiologies (samples obtained within 48 hr after hospital admission).

MAIN OUTCOMES AND MEASURES: Primary outcome measure was mortality. Secondary outcome measures were PICU-free days at day 28 and hospital length of stay.

RESULTS: In cohort 1 (n = 59), nonsurvivors more frequently had A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 levels above the detection limit than survivors at admission to PICU (8/11 [73%] and 6/23 [26%], respectively; p = 0.02) and at t = 24 hours (2/3 [67%] and 3/37 [8%], respectively; p = 0.04). In cohort 2 (n = 240), A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 levels in patients within 48 hours after hospital admission were more frequently above the detection limit than in healthy controls (110/240 [46%] and 14/64 [22%], respectively; p = 0.001). Nonsurvivors more often had detectable A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 levels than survivors (16/21 [76%] and 94/219 [43%], respectively; p = 0.003), which was mostly attributable to patients with Neisseria meningitidis.

CONCLUSIONS AND RELEVANCE: In children with bacterial infection, detection of A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 within 48 hours after hospital admission is associated with death, particularly in meningococcal sepsis. Future studies should confirm the prognostic value of A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 and should study pathophysiologic mechanisms.

KEY WORDS: A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 protein; bacterial infections; biomarkers; inflammation; mortality; sepsis

P revalence and outcome of bacterial infections are determined by host (e.g., genetic predisposition, immune response to bacteria), pathogen, and healthcare system factors (1). The EUropean Childhood Life-threatening Infectious Diseases Study (EUCLIDS) aims to identify genetic

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factors and biological pathways associated with susceptibility and/or severity of life-threatening bacterial infections (2–4). Preliminary EUCLIDS genetic studies in meningococcal sepsis patients identified a SNP in A Disintegrin and Metalloproteinase with Thrombospondin Motifs-1 (ADAMTS-1; rs9975310) to be associated with disease severity, although this association did not reach genome-wide significance (unpublished data). Furthermore, animal studies showed that ADAMTS-1 is increased in the host inflammatory response, and therefore we hypothesize that ADAMTS-1 plays a role in the pathogenesis of invasive infection (5–7). Studies on ADAMTS-1 in sepsis, either adult or pediatric, are currently lacking.

The ADAMTS family includes 19 proteases with a variety of functions, for example, in coagulation and inflammation (8–11). ADAMTS-13, the von Willebrand factor (vWF)-cleaving protease, cleaves ultra large prothrombotic multimeric vWF into an optimal size for normal coagulation (12) and is the most extensively studied ADAMTS protease in sepsis. Previous studies demonstrated that decreased ADAMTS-13 levels, presumably leading to increased formation of thrombi, are associated with more severe disease and poor outcome (13, 14).

Other ADAMTS-proteins have not been studied in sepsis yet despite animal studies hinting toward an important role in inflammation and sepsis. ADAMTS-1 is an inhibitor of angiogenic activity, is associated with acute inflammatory processes, and is involved in the process of extracellular matrix damage and repair (7, 15). In rats and mice, a dramatic increase of ADAMTS-1 was detected after lipopolysaccharide (LPS) induced systemic inflammation, suggesting that the ADAMTS-1 gene is an inflammation-associated gene (5–7). An immunomodulatory role for ADAMTS-1 is also indicated by the pro-inflammatory phenotype observed in ADAMTS-1-deficient mice (16).

We studied ADAMTS-1 serum protein levels in pediatric sepsis and studied the association with mortality, illness severity, coagulation, and infecting pathogen.

**MATERIALS AND METHODS**

This study comprises data from two independent cohorts; a single-center cohort of children admitted to PICU with meningococcal sepsis (cohort 1) and an international, multicenter cohort of children admitted to hospital with invasive bacterial infections of differing etiologies (cohort 2).

### Cohort 1

Children 1 month to 18 years old with meningococcal sepsis presenting to the PICU of Erasmus MC-Sophia Children’s Hospital between October 1991 and February 2000 were prospectively enrolled in meningococcal sepsis studies (17–19). All patients fulfilled internationally agreed criteria for sepsis with petechial rash and/or purpura (20). Blood samples were collected at admission to PICU, at 24 hours, and at 1 month after PICU admission.

Serum samples were processed on ice and stored at −80°C until analysis. In remaining serum samples available from these studies, we measured ADAMTS-1 levels using a commercially available human enzyme-linked immunosorbent assay (ELISA) kit as described by the manufacturer (ADAMTS-1 ELISA kit, MBS2021525; MyBioSource, San Diego, CA). The lower limit of detection (LLOD) of this assay was 1.6 ng/mL (1,600 pg/mL). ADAMTS-1 levels measured below the LLOD were considered 1.6 ng/mL.

The samples obtained 1 month after PICU admission were considered as convalescent samples.

### Cohort 2

Children suspected of community-acquired bacterial infection at hospital admission were prospectively enrolled between July 2012 and December 2016. This multicenter cohort study (EUCLIDS) involves 195 hospitals from 10 countries. Detailed information on consortium and enrollment strategy has been published elsewhere (2, 3). Patients were recruited as early as possible in the illness within a time window from admission to hospital to the time when microbiology results became available.

For this laboratory study, we selected children 1 month to 18 years old recruited in five countries (United Kingdom, Spain, Austria, The Netherlands, and Switzerland) with an invasive infection caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, or group A streptococcus, from whom serum samples obtained within 48 hours after hospital admission were available. Invasive bacterial infection was defined as isolation by culture or polymerase chain reaction of a bacterial organism from a normally sterile site. We considered blood, cerebrospinal fluid, urine, bronchoalveolar lavage, joint aspirate, abscess aspirate, intraoperative swabs, and pleural aspirate as sterile sites. Positive cultures from sites
such as endotracheal tube aspirate, nasopharyngeal aspirate, throat/nasal swabs, and wounds were not considered as sterile sites.

ADAMTS-1 levels were measured with a custom-made Luminex assay based on a capture antibody, detection antibody, and recombinant human ADAMTS-1 (ADAMTS-1 DuoSet ELISA assay; R&D Systems, Abingdon, United Kingdom). This Luminex assay, being a far more sensitive assay than the ELISA used for cohort 1, had a LLOD of 7.0 pg/mL.

For comparison, EUCLIDS recruited healthy controls from whom serum was obtained prior to elective surgical procedures. The controls did not have any underlying inflammatory comorbidity.

**Ethical Aspects**

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The Erasmus MC—meningococcal sepsis study was approved by the ethical committee of Erasmus MC (MEC-2015-497), and the EUCLIDS study protocol was approved by at least one ethical review board in every participating country (Coordinating Center Research Ethics Committee reference: 11/LO/1982). Written informed consent was obtained from parents or legal guardians.

**Clinical Data Collection**

Data for both cohorts were collected prospectively. Illness severity and risk of mortality were assessed by the Pediatric Risk of Mortality (PRISM) score (21), Pediatric Index of Mortality 2 (PIM 2) (22), need for ventilation and/or inotropes, predicted death based on the base excess and platelet count at presentation score (23), predicted death based on the Rotterdam score (17), and disseminated intravascular coagulation (DIC) score (24). Coagulation and inflammation markers were measured for clinical reasons or measured as requirement for other meningococcal sepsis studies to which patients had been recruited (17, 18, 25). For the multicenter EUCLIDS study, monthly telephone conferences, biannual meetings, clinical protocols including case definitions, data audits, and monitoring ensured uniform procedures among study sites.

**Outcome Measures**

The primary outcome measure was mortality. Patients were classified as deceased if death occurred during hospital stay. Secondary outcome measures were PICU-free days at day 28 (days alive and free from the need for intensive care) and hospital length of stay. PICU-free days in patients who died were considered zero.

**Statistical Analysis**

Categorical variables are presented as counts (percentages). We used the chi-square test (or Fisher exact test in case the number of events in one group was < 5) to compare frequency distributions between two categorical variables. Continuous variables with normal distribution are presented as mean (± sd); non-normally distributed variables are reported as median (interquartile range [IQR]). We tested differences between groups with analysis of variance or Kruskal-Wallis and Student t test or Mann-Whitney U test, as appropriate. In the cohort 1, Friedman tests were used to compare ADAMTS-1 levels between three time points. Correlations between ADAMTS-1 level and secondary outcome measures, illness severity, coagulation markers, and inflammatory markers were assessed by Spearman rank correlation. Post hoc Bonferroni correction for multiple testing was applied. Statistical analyses were performed with SPSS Version 21 (Armonk, NY). Graphs were created with GraphPad Prism 8.4.0 (GraphPad Software, Inc.). A p value of less than 0.05 was considered statistically significant.

**RESULTS**

**Cohort 1**

We included 59 children admitted to PICU with meningococcal sepsis, of whom 11 (19%) died, who had 109 samples available for ADAMTS-1 measurements. Patient characteristics are shown in Table 1.

Because ADAMTS-1 levels of 90 of 108 samples (83%) were below the LLOD of the assay (1.6 ng/mL), we compared the number of samples with detectable ADAMTS-1 (designated as ADAMTS-1 ≥ 1.6 ng/mL) with the number of samples with undetectable ADAMTS-1. At admission to PICU, ADAMTS-1 was detectable in 14 of 34 patients (41%), which was more frequent than in patients at t = 24 hours (5/40 [12%]; p = 0.005) and at t = 1 month (0/35 [0%]; p < 0.001) (Table 2). Nonsurvivors more frequently had detectable ADAMTS-1 levels compared with survivors at admission to PICU (nonsurvivors 8/11 [73%], survivors 6/23 [26%]; p = 0.02) and at t = 24 hours (nonsurvivors 2/3 [67%], survivors 3/37 [8%]; p = 0.04).
| Variable                          | Cohort 1 Patients (n = 59) | Cohort 2 Patients (n = 240) | Cohort 2 Controls (n = 64) | p     |
|----------------------------------|---------------------------|----------------------------|---------------------------|-------|
| Male                             | 35 (59%)                  | 131 (55%)                  | 34 (53%)                  | NS    |
| Age                              | 3.0 yr (1.8–9.7 yr)        | 3.4 yr (15 mo–9.2 yr)      | 5.4 yr (2.8–12.4 yr)      | < 0.01|
| Ethnicitya                       |                           |                           |                           | NS    |
| African/North African            | –                         | 12 (5%)                   | 8 (13%)                   |       |
| Asian                            | –                         | 13 (6%)                   | 4 (6%)                    |       |
| European                         | –                         | 185 (81%)                 | 47 (75%)                  |       |
| Meso/South American              | –                         | 4 (2%)                    | 0 (0%)                    |       |
| Middle Eastern                   | –                         | 3 (1%)                    | 2 (3%)                    |       |
| Other/mixed                      | –                         | 12 (5%)                   | 2 (3%)                    |       |
| Number of underlying conditions  |                           |                           |                           | < 0.01|
| None                             | –                         | 138 (58%)                 | 25 (39%)                  |       |
| ≥ 1                              | –                         | 102 (42%)                 | 39 (61%)                  |       |
| Immunizations up to dateb        | –                         | 178 (95%)                 | 58 (95%)                  | NS    |
| Illness severity                 |                           |                           |                           |       |
| Sepsis                           | –                         | 159 (66%)                 |                           |       |
| PICU admission                   | 59 (100%)                 | 177 (74%)                 |                           |       |
| Need for inotropesc              | 42 (95%)                  | 107 (53%)                 |                           |       |
| Days on inotropes                | –                         | 3 (2–5)                   |                           |       |
| Need for invasive ventilationd   | 31 (60%)                  | 106 (52%)                 |                           |       |
| Days on invasive ventilation     | –                         | 4 (3–7)                   |                           |       |
| Need for extracorporeal membrane oxygenationc | – | 4 (3%) | | |
| Pediatric Risk of Mortality (21) scored | 20 (14–26) | 11 (7–16) | | |
| Pediatric Index of Mortality 2 (22) scorec | – | 3.5% (0.8–11.6) | | |
| Predicted death rate based on the base excess and platelet count at presentation score (23)d | 6.1 (3.4–19.8) | – | | |
| Predicted death rate based on the Rotterdam score (17)i | 12.2 (1.6–77.0) | – | | |
| Lactate (mmol/L)d                | 4.5 (3.3–6.5)             | –                         |                           |       |
| DIC scoree                       | 5 (4–7)                   | 2 (0–2)                   |                           |       |
| Presence of DIC (DIC score ≥ 5)i | 22 (61%)                  | 18 (10%)                  |                           |       |

(Continued)
TABLE 1. (Continued).
Baseline Characteristics Cohort 1 and Cohort 2

| Variable          | Cohort 1 Patients (n = 59) | Cohort 2 Patients (n = 240) | Cohort 2 Controls (n = 64) | p  |
|-------------------|---------------------------|-----------------------------|----------------------------|----|
| Outcome           |                           |                             |                            |    |
| PICU-free days at day 28 (d) supk | 23 (13–25) | 23 (19–25) | | 0.02 a |
| Hospital length of stay (d) supl | 7 (3–12) | 10 (6–17) | | 0.02 b |
| Death             | 11 (19%)                  | 21 (9%)                     |                            |    |

DIC = disseminated intravascular coagulation (24, 26), NS = not significant.
*Ethnicity data were available for 229/240 patients and 63/64 controls.
*Immunization data were available for 188/240 patients and 61/64 controls.
*Data on inotropes were available for 44/59 patients in cohort 1 and 202/240 patients in cohort 2.
*Data on invasive ventilation were available for 52/59 patients in cohort 1 and 203/240 patients in cohort 2.
*Data on extracorporeal membrane oxygenation were available for 161/240 patients.
*Pediatric Risk of Mortality score (21) was available for 50/59 patients in cohort 1 and 150/240 patients in cohort 2.
*Pediatric Index of Mortality 2 score (22) was available for 177/240 patients.
*Predicted death rate based on the base excess and platelet count at presentation score (23) and lactate were available for 52/59 patients.
*Predicted death rate based on the Rotterdam score (17) was available for 48/59 patients.
*Data on DIC were available for 36/59 patients in cohort 1 and 187/240 patients in cohort 2.
*Data on PICU-free days at day 28 were available for 59/59 patients in cohort 1 and 177/177 PICU patients in cohort 2.
*Data on hospital length of stay were available for 53/59 patients in cohort 1 and 240/240 patients in cohort 2.

Values are reported as counts (percentages) or medians (interquartile ranges) unless stated otherwise. Dashes indicate data is on this variable is not available and no statistical test has been done.

TABLE 2.
Cohort 1; A Disintegrin and Metalloproteinase With Thrombospondin Motifs-1 Levels in Survivors and Nonsurvivors at Admission to PICU, at t = 24 Hours and at t = 1 Month

| Time Point      | All Patients (n = 59) | Survivors (n = 48) | Nonsurvivors (n = 11) | p  |
|-----------------|-----------------------|--------------------|-----------------------|----|
| PICU admission  | n = 34, 1.6 (1.6–2.1) | n = 23, 1.6 (1.6–1.6) | n = 11, 2.0 (1.6–3.1) | 0.02 a |
| n < 1.6         | n = 20 (59%)          | n = 17 (74%)       | n = 3 (27%)           | 0.02 b |
| n > 1.6         | n = 14 (41%)          | n = 6 (26%)        | n = 8 (73%)           |      |
| t = 24 hr       | n = 40, 1.6 (1.6–1.6) | n = 37, 1.6 (1.6–1.6) | n = 3, 2.1 (1.6–3.3)  | Not significant a |
| n < 1.6         | n = 35 (88%)          | n = 34 (92%)       | n = 1 (33%)           | 0.04 b |
| n > 1.6         | n = 5 (12%)           | n = 3 (8%)         | n = 2 (67%)           |      |
| t = 1 mo        | n = 35, 1.6 (1.6–1.6) | n = 35, 1.6 (1.6–1.6) | n = 0                 |      |
| n < 1.6         | n = 35 (100%)         | n = 35 (100%)      | n = 0                 |      |
| n > 1.6         | n = 0                 | n = 0              | n = 0                 |      |

*Mann-Whitney U test.
*Fisher exact test.

A Disintegrin and Metalloproteinase With Thrombospondin Motifs-1 levels are presented as median (interquartile range).
For each time point, we additionally analyzed the number of samples below and above the lowest level of detection (1.6 ng/mL). Dashes indicate data is on this variable is not available and no statistical test has been done.
Median ADAMTS-1 level at admission to PICU (n = 34; 1.6 ng/mL [IQR, 1.6–2.1 ng/mL]) did not differ from ADAMTS-1 level at t = 24 hours (n = 40; 1.6 ng/mL [IQR, 1.6–1.6 ng/mL]) or at t = 1 month (n = 35; 1.6 ng/mL [IQR, 1.6–1.6 ng/mL]; Friedman test \(p = 0.37\) (Table 2). At admission to PICU, ADAMTS-1 levels in nonsurvivors (n = 11; 2.0 ng/mL [IQR, 1.6–3.1 ng/mL]) were significantly higher than in survivors (n = 23; 1.6 ng/mL [IQR, 1.6–1.6 ng/mL]; \(p = 0.02\)). Although numbers were low (n = 40), after 24 hours, there still was a trend for higher ADAMTS-1 levels in nonsurvivors (n = 3; 2.1 ng/mL [IQR, 1.6–3.3 ng/mL]) compared with survivors (n = 37; 1.6 ng/mL [IQR, 1.6–1.6 ng/mL]; \(p = 0.09\)).

ADAMTS-1 level at admission to PICU was not significantly correlated to PICU-free days at day 28 (\(r = -0.31; p = 0.08\)) and hospital length of stay (\(r = -0.48; p = 0.01\)) nor did they correlate with illness severity, coagulation markers, or inflammatory markers (Supplemental Digital Content, http://links.lww.com/CCX/A845).

Cohort 2

We included 240 children with an invasive infection caused by *N. meningitidis* (n = 83), *S. pneumoniae* (n = 63), *S. aureus* (n = 50), or group A streptococcus (n = 44), of which 21 children died (9%). Additionally, we included 64 controls (age ranged from 1 mo to 18 yr). Baseline characteristics are shown in Table 1, and baseline characteristics per pathogen are shown in Table 3.

ADAMTS-1 level within 48 hours after admission to hospital was more frequently detectable in patients (110/240 [46%]) compared with controls (14/64 [22%]; \(p = 0.001\)). Furthermore, although median values were similar, ADAMTS-1 level analyzed by the rank-sum test differed between patients and controls (patients: median 7.0 pg/mL [IQR, 7.0–118 pg/mL]; controls: median 7.0 pg/mL [IQR, 7.0–7.0 pg/mL]; \(p < 0.001\)) (Fig. 1). The elevation in ADAMTS-1 was more pronounced in PICU patients (n = 177; median, 11.7 pg/mL [IQR, 7.0–166 pg/mL]) than in non-PICU patients (n = 63; median, 7.0 pg/mL [IQR, 7.0–18.5 pg/mL]; \(p = 0.001\)).

Detection of ADAMTS-1 was more frequent in patients with *N. meningitidis* (41/83 [49%]; \(p = 0.001\)), *S. aureus* (21/50 [42%]; \(p = 0.02\)), and group A streptococcus (25/44 [57%]; \(p < 0.001\)) compared with controls (14/64 [22%]). Detection in patients with *S. pneumoniae* (23/63 [37%]; \(p = 0.07\)) did not differ from controls. ADAMTS-1 level per pathogen group is depicted in Figure 1.

In samples taken within 48 hours after hospital admission, ADAMTS-1 was detected in nonsurvivors (16/21 [76%]) more frequently than in survivors (9/4/219 [43%]; \(p = 0.003\)). Furthermore, ADAMTS-1 levels in nonsurvivors (median, 260 pg/mL [IQR, 45–1,548 pg/mL]) were higher compared with survivors (median, 7.0 pg/mL [IQR, 7.0–96 pg/mL]; \(p < 0.001\)) (Fig. 2A).

This was attributable to patients with *N. meningitidis* (detectable nonsurvivors 7/7 [100%], detectable survivors 34/76 [45%]; \(p = 0.005\)). Median ADAMTS-1 level also differed between meningococcal infection nonsurvivors and survivors (nonsurvivors: median, 687 pg/mL [IQR, 120–4,108 pg/mL]; survivors: median, 7.0 pg/mL [IQR, 7.0–111 pg/mL]; \(p < 0.001\)) (Fig. 2B). In children with meningococcal infections, ADAMTS-1 level at admission to hospital was correlated to PICU-free days at day 28 (\(r = -0.54; p < 0.001\), PRISM score (\(r = 0.47; p < 0.001\)), DIC score (\(r = 0.42–p < 0.001\)), and plasmin-ogen activator inhibitor-1 (PAI-1) (\(r = 0.42; p < 0.001\)), but not to hospital length of stay (\(r = 0.12; p = 0.30\)).

ADAMTS-1 detection did not differ significantly between survivors and nonsurvivors of infections with *S. pneumoniae* (detectable nonsurvivors 3/7 [43%], detectable survivors 20/56 [36%]; \(p = 0.71\)), *S. aureus* (detectable nonsurvivors 3/4 [75%], detectable survivors 18/46 [39%]; \(p = 0.16\)), and group A streptococcus (detectable nonsurvivors 3/3 [100%], detectable survivors 22/41 [54%]; \(p = 0.12\)). ADAMTS-1 levels in survivors and nonsurvivors per pathogen group are depicted in Figure 2B.

With regard to secondary outcome measures, ADAMTS-1 levels at admission to hospital were strongly correlated to PICU-free days at day 28 (\(r = -0.36; p < 0.001\), PRISM score (\(r = 0.37; p < 0.001\)), DIC score (\(r = 0.27; p < 0.001\)), need for invasive ventilation (\(r = 0.27; p < 0.001\)), platelets (\(r = -0.30; p < 0.001\)), protein C (\(r = -0.24; p < 0.001\)), and PAI-1 (\(r = 0.33; p < 0.001\)), but less strongly with hospital length of stay (\(r = -0.13; p = 0.05\)) (Supplemental Digital Content, http://links.lww.com/CCX/A845).

Thus, in both cohorts, ADAMTS-1 in nonsurvivors was more frequently detectable and showed a
### TABLE 3.
Baseline Characteristics Cohort 2 by Pathogen

| Variable                                      | All Patients (n = 240) | Neisseria meningitidis (n = 83) | Streptococcus pneumoniae (n = 63) | Staphylococcus aureus (n = 50) | Group A Streptococcus (n = 44) | p  |
|-----------------------------------------------|------------------------|----------------------------------|-----------------------------------|-------------------------------|-----------------------------|----|
| Gender, male                                  | 131 (55%)              | 50 (60%)                         | 39 (62%)                         | 25 (50%)                      | 17 (39%)                    | NS |
| Age                                           | 3.4 yr (15 mo–9.2 yr)  | 1.8 yr (8 mo–5.3 yr)             | 2.6 yr (16 mo–5.6 yr)            | 9.9 yr (4.2 yr–13.1 yr)       | 3.7 yr (18 mo–7.9 yr)       | <0.001 |
| Ethnicity<sup>a</sup>                          |                        |                                  |                                   |                               |                             |    |
| African/North African                          | 12 (5%)                | 2 (3%)                           | 4 (7%)                            | 4 (9%)                        | 2 (5%)                      | NS |
| Asian                                         | 13 (6%)                | 1 (1%)                           | 2 (3%)                            | 4 (9%)                        | 6 (14%)                     |    |
| European                                       | 185 (81%)              | 69 (89%)                         | 49 (79%)                          | 35 (76%)                      | 32 (74%)                    |    |
| Meso/South American                            | 4 (2%)                 | 0 (0%)                           | 2 (3%)                            | 2 (4%)                        | 0 (0%)                      |    |
| Middle Eastern                                 | 3 (1%)                 | 2 (3%)                           | 0 (0%)                            | 1 (2%)                        | 0 (0%)                      |    |
| Other/mixed                                    | 12 (5%)                | 4 (5%)                           | 5 (8%)                            | 0 (0%)                        | 3 (7%)                      |    |
| Number of underlying conditions                |                        |                                  |                                   |                               |                             | NS |
| None                                          | 138 (58%)              | 56 (68%)                         | 33 (52%)                          | 23 (46%)                      | 26 (59%)                    |    |
| ≥ 1                                           | 102 (42%)              | 27 (32%)                         | 30 (48%)                          | 27 (54%)                      | 18 (41%)                    |    |
| Immunizations up to date<sup>b</sup>           | 178 (95%)              | 63 (96%)                         | 49 (96%)                          | 32 (89%)                      | 34 (97%)                    | NS |
| Illness severity                               |                        |                                  |                                   |                               |                             |    |
| Sepsis                                        | 159 (66%)              | 65 (78%)                         | 36 (57%)                          | 25 (50%)                      | 33 (75%)                    | <0.01 |
| PICU admission                                 | 177 (74%)              | 73 (88%)                         | 40 (64%)                          | 24 (48%)                      | 40 (91%)                    | <0.001 |
| Need for inotropes<sup>c</sup>               | 107 (53%)              | 52 (71%)                         | 12 (26%)                          | 15 (36%)                      | 28 (70%)                    | <0.001 |
| Days on inotropes                              | 3 (2–5)                | 3 (2–4)                          | 4 (1–6)                           | 3 (3–7)                       | 4 (2–5)                     | NS |
| Need for invasive ventilation<sup>d</sup>      | 106 (52%)              | 45 (62%)                         | 18 (38%)                          | 13 (30%)                      | 30 (75%)                    | <0.001 |
| Days on invasive ventilation                  | 4 (3–7)                | 5 (3–6)                          | 3 (2–9)                           | 4 (3–19)                      | 4 (2–8)                     | NS |
| Need for extracorporeal membrane oxygenation<sup>e</sup> | 4 (3%)                | 1 (2%)                           | 0 (0%)                            | 2 (9%)                        | 1 (3%)                      | NS |
| Pediatric Risk of Mortality (21) score<sup>f</sup> | 11 (7–16)              | 13 (7–16)                        | 10 (6–17)                         | 11 (7–19)                     | 12 (7–15)                   | NS |
| Pediatric Index of Mortality 2 (22) score<sup>g</sup> (predicted death, %) | 3.5% (0.8–11.6)       | 3.5% (0.8–13.0)                  | 2.7% (0.8–9.3)                   | 2.6% (0.8–4.5)                | 6.2% (1.0–13.0)             | NS |
| DIC score<sup>h</sup>                          | 2 (0–2)                | 2 (2–4)                          | 2 (0–2)                           | 2 (0–2)                       | 2 (0–2)                     | <0.01 |
| Presence of DIC (DIC score ≥ 5)<sup>h</sup>   | 18 (10%)               | 10 (14%)                         | 2 (4%)                            | 3 (8%)                        | 3 (9%)                      | NS |

(Continued)
higher level than in survivors. And in cohort 2 only, ADAMTS-1 levels were correlated to PICU-free days at day 28.

**DISCUSSION**

This study is the first to show that ADAMTS-1 serum levels are elevated in children admitted to hospital with bacterial infection and sepsis. Importantly, our study demonstrates that in nonsurvivors ADAMTS-1 serum levels were more often detectable than in survivors, especially in patients with *N. meningitidis* disease. Additionally, ADAMTS-1 levels were correlated to PICU-free days and other markers for illness severity.

Our findings are in line with observations in experimental sepsis models where plasma levels of ADAMTS-1 in rats increased after injection with *Escherichia coli* LPS (5). Furthermore, interleukin (IL)-1β, a pro-inflammatory cytokine implicated in pediatric sepsis (27), was found to induce ADAMTS-1 production in human decidual stromal cells in vitro (28). Apart from sepsis-induced inflammation, inflammation related to nerve injury and cancer is also associated with increased ADAMTS-1 production (7, 29).

The role of ADAMTS-1 in the pathophysiology of bacterial infection, and in particular meningococcal disease, is mostly unclear so far. The association of ADAMTS-1 on sepsis mortality may be due to interference with vascular endothelial growth factor (VEGF) and VEGF receptor-2 signaling that have been involved in the pathophysiology of sepsis (30–35). ADAMTS-1 binds VEGF and blocks the VEGF receptor-2 (36), thus potentially contributing to sepsis-induced organ dysfunction (31). Furthermore, an immune-modulatory/
suppressive role for ADAMTS-1 has also been proposed (16), possibly resulting in high levels of anti-inflammatory cytokines (e.g., IL-10, IL-1 receptor antagonist, and soluble tumor necrosis factor receptors) that are associated with sepsis mortality (37, 38). In line with these hypotheses, we found that a higher ADAMTS-1 serum level was associated with increased mortality. Although future studies should further elucidate the pathophysiological role of ADAMTS-1, our current data indicate that ADAMTS-1 can be part of the inflammatory response to pediatric sepsis.

When comparing ADAMTS-1 levels in different pathogens, our findings in nonsurvivors versus survivors were most pronounced in patients with *Neisseria meningitidis* and partly in *Group A streptococcus* infections. Although mortality across the pathogen groups was comparable, patients with *N. meningitidis* and *group A streptococcus* infections more often had sepsis, including the need for inotropes and invasive ventilation. The systemic inflammatory response in these patients might have contributed to higher ADAMTS-1 levels. Additionally, pathogen-specific properties interfere with the host response to infection (39, 40). *N. meningitidis* and/or *group A streptococcus* could possess properties interacting with ADAMTS-1.

A major strength of our study is that we used two independent cohorts that both revealed comparable changes in ADAMTS-1 serum level. Because the assays in both cohorts differ, we are not able to compare absolute values of ADAMTS-1. Other strengths of our study were that we examined ADAMTS-1 levels in sepsis caused by different pathogens and correlations of ADAMTS-1 with illness severity, coagulation, and inflammatory markers. Our study is possibly limited by the long-time storage of samples from cohort 1. The stability of ADAMTS-1 proteins in stored samples...
is unknown. However, if samples would be affected, we assume that all samples would be affected equally. Also, we did not compare ADAMTS-1 levels measured in cohort 1 with controls. We considered convalescent samples (taken at 1 mo after PICU admission) as appropriate control for the initial measurements, but ADAMTS-1 levels after critical illness are unknown.

Comparisons between cohort 1 and cohort 2 are also limited by the variation in time from hospital onset to blood sampling. Cohort 1 collected samples at admission to PICU, at 24 hours, and at 1 month after PICU admission, while cohort 2 included all blood samples taken within 48 hours after hospital admission. Because the course of ADAMTS-1 protein levels in human sepsis is unknown, we do not know the impact of clustering of samples from cohort 2 for analysis. However, ADAMTS-1 level was not correlated to the time interval between hospital admission and the time of blood sample (data not shown).

CONCLUSIONS

Detectable ADAMTS-1 is associated with disease severity in sepsis, particularly in meningococcal sepsis, with higher ADAMTS-1 levels in nonsurvivors than in survivors. Future studies should confirm the prognostic value of ADAMTS-1 in adult sepsis and should study possible pathophysiologic mechanisms to identify potential therapeutic targets.

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