Bloody Diarrhea and Shiga Toxin–Producing Escherichia coli Hemolytic Uremic Syndrome in Children: Data from the ItalKid-HUS Network

Bloody Diarrhea and STEC-HUS in Children: Data from the ItalKid-HUS Network

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Abbreviations

~~BD: bloody diarrhea~~

CI: confidence interval

Eae: intimin

HUS: hemolytic uremic syndrome

ipaH: invasion plasmin antigen H gene

IQR: interquartile range

LPS: lipopolysaccharide

STEC: shigatoxin-producing *Escherichia coli*

Stx: shiga-toxin

TMA: thoromboticmicroangiopathy

VTEC :verocytotoxin-producing *Escherichia coli*

Yr: year

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Data Sharing Statement

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Deidentified individual participant data will not be made available.

~~**Contributors' statement**~~

~~Dr. Ardissino, Dr. Arghittu, Dr. Capone, Dr. Tel, Dr. Testa, Dr. Paglialonga, Dr. Brigotti, Dr. Baldioli, Dr. Pagani, Dr. Ceruti, Dr. Tommasi, Dr. Possenti and Dr. Cresseri conceptualized and designed the study, participated in data collection, drafted the initial manuscript, and reviewed and revised the manuscript.~~

~~Dr. Vignati, Dr. Masia, Dr. Colombo, Dr. Daprai, Dr. Dodaro, Dr. Luini and Dr. Picicco performed laboratory tests and data collection and carried out analysis and revised the manuscript~~

~~Dr. Consonni and Dr. Montini critically reviewed the manuscript for important intellectual content.~~

~~All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.~~

~~Abstract~~

Objective: To analyze the results of an enhanced laboratory-surveillance protocol for bloody diarrhea ~~(BD)~~ aimed at identifying children with *Shiga Toxin-Producing Escherichia coli* (STEC) infection early in the course of the disease towards the early identification and management of patients with Hemolytic Uremic Syndrome (HUS).

~~Methods~~**Study design:** The study (2010-2019) involved a referral population of 2.3 million children. Stool samples of patients with BD were screened for Shiga Toxin (Stx) genes. Positive patients were rehydrated and monitored for hemoglobinuria until diarrhea resolved or STEC-HUS was diagnosed.

Results: A total of 4767 children were screened; 214(4.5%) were positive for either Stx1(29.0%) or Stx2(45.3%) or both Stx1+2(25.7%); 34~~. Thirty four~~ patients (15.9%) developed STEC-HUS (0.71% of BDs). Hemoglobinuria was present in all patients with HUS. Patients with Stx2 alone showed a higher risk of STEC-HUS (23.7% vs~~.~~12.7%) ~~while~~ and none of the patients with Stx1 alone developed HUS. During the sameperiod of time 95 other patients were diagnosed STEC-HUS but were not captured by the screening program (26 had non-BD and 11 came from areas not covered by the screening program and 58 had not been referred to the screening program although they did meet the inclusion criteria). At HUS presentation, serum creatinine of patients identified by screening was significantly lower compared ~~to~~ with that of the remaining patients (median: 0.9 vs 1.51 mg/dL).

Discussion: Nearly 1% of children with BD developed STEC-HUS and its diagnosis was anticipated by the screening program for Stx. The screening of BD for Stx is recommended and monitoring patients carrying Stx2 with urine dip-stick for hemoglobinuria is suggested to identify the renal complication as early as possible.

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~~**Background**~~

The epidemiology of acute bloody diarrhea (BD) in children is not clearly known both in high and low-middle income countries [1,2]. What is known is that a wide array of pathogens can cause BD in children with varying incidence by geography, season, age, socioeconomic conditions and hosts' immune status [3-9].

In Western countries culture-proven BD is mostly due to *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia* and *Escherichia coli* especially Shiga toxin-producing *Escherichia coli* (STEC) [10-16].

STEC infections can be complicated by the hemolytic uremic syndrome (HUS) [17] that, besides being the most common thrombotic microangiopathy (TMA) in children, is the leading cause of acute kidney injury in children beyond the neonatal age [18]. STEC-HUS is characterized by platelet consumption, mechanical non-immune mediated hemolysis and renal, as well as multi-organ, damage with severe and life-threatening consequences.

Detailed knowledge of the local epidemiology is of paramount importance for identifying new and effective prevention strategies as well as for driving the optimal diagnostic approach. Moreover, the patient's early clinical management can be modified by knowledge of epidemiological data (virulence profiling and risk of complications).

~~The present paper~~We describe~~s~~ the results obtained from the 10 year~~s~~ experience with a centralized screening program of BD for *Shiga Toxin (Stx)*in children that was promoted in a large area with over 12 million general population. The study presents the general epidemiology of *Stx*+BD in children with special regard to HUS development and the impact of the screening program in anticipating the diagnosis and the management of HUS.

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Methods

~~Study setting and design~~

The present analysis includes all tested children (up to age 20 years of age) with BD obtained from 2010 through 2019 within a network of 63 pediatric units in Northern Italy (ItalKid-HUS Network), with a referral pediatric population of 2.3 million. We present all STEC-HUS cases diagnosed during the analyzed period including those not screened prior to the diagnosis of HUS, either because they were not tested (n: 58) due to the rapidcourse of the diseaseor because they came from areas not covered by the screening program (n: 11) or because they did not exhibit bloody diarrhea at all (n: 26) (Figure 1).

The network was developed for the identification of STEC infections aimed at the early diagnosis and management of STEC-HUS.

Exclusion criteria were history of chronic diarrhea due to any cause.

Stool specimens (occasionally, fecal or rectal swabs) were obtained either from the emergency department or upon patient's admission and only one sample was recorded for each patient. Samples were delivered at room temperature and accepted 24 hours a day /7 days a week and the results were made available during the subsequent day since sample receipt.

All the patients/parents were informed about the investigations and gave their written consent to the diagnostic procedures. The study received the approval by the Ethical Committee of our Institution.

Objectives and End-points

The present study was carried out on an intention-to-diagnose basis. The study was aimed at anticipating the diagnosis and the management of STEC-HUS while evaluating the prevalence of STEC-infection among children with BD. Primary end-points of the study were the measurement of the proportion of Stx positive patients among children with acute BD, the distribution of Stxs and STEC serotypes involved. Secondary end-point was the comparison of laboratory (serum creatinine,

platelet counts and hemoglobin) at presentation and the outcomes of HUS in patients identified by screening and/or diagnosed with HUS (with or without prodromal BD).

Definitions

BD was defined as: “acute (<10 days) diarrhea with visible blood in at least one bowel movement either seen by health professionals or reported by caregivers”.

STEC-HUS was defined as the concomitant presence of platelet consumption (platelet count<150,000/mm^3 or more than 50% acute reduction of plateletcount), non-immune mediated (Coombs negative) hemolysis (anemia or undetectabl ehaptoglobin or LDH above upper limit of normal) and renal damage (serum creatinine above upper normal limit for age and ~~gender~~ sex or proteinuria or hematuria) in a patient with evidences of STEC infection (Stx genes in stool or anti-LPS positivityagainst the “top 6” serotypes).“Top 6” serotypes are: O157, O26, O103, O111, O145 and O104 [19].Cases with negative Stx and negative anti-LPS, were further investigated to exclude complement dysregulation. The same was done in cases of atypical course and/or with poor outcome. Positive urine dipstick or urinalysis for hemoglobinuria was defined as ≥ +(small) or ≥ 20 mg/mL, respectively. Positive urine dipstick or urinalysis for hematuria (presence of RBCs) wasdefinedas ≥ + (small) or ≥~~5~~ RBCs/HPF, respectively."

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Laboratory procedures

The biological samples are centralized at our Center where the test for the detection of STEC-related genes was performed using the Reverse Dot Blot Assay (Genotype EHEC (Arnika)) until 2018 and subsequently a Real-Time PCR (RIDA Gene-Relab).

In detail, 50 µL of feces or rectal swabs are inoculated on the MacConkey broth and incubated at 37° C for 18-24 hours. DNA from bacteria is extracted (Gentra Puregene blood kit) and quantified (NanoDrop 1000 spectrophotometer). Reverse Dot Blot (Genotype EHEC-Arnika) was used to identify the following genes: *Stx1, Stx2, eae* and *ipaH*. The target DNA was amplified with 5

'biotinylated primers and hybridized to specific oligonucleotides immobilized on the membrane strips. Hybridization is detected by adding streptavidin-horseradish peroxidase to the membrane hence obtaining a colorimetric reaction.

Since 2018, STEC-related genes were detected by multiplex Real-Time PCR performed by the RIDA Gene-EHEC/EPEC (R-biopharm) screening kit. If the screening was positive for *Stx*, a second multiplex Real-Time PCR was performed using the RIDA Gene E. coli Stool Panel I kit (R-biopharm) to discriminate between *Stx1* and *Stx2* genes. The amplified targets are revealed with probes marked at the ends, respectively, with a quencher on one side and with a fluorescent dye (fluorophore) on the other. In the presence of a target, the probes hybridize with amplicons. The main serotypes of STEC were identified using a Real Time multiplex PCR for the serogroups most frequently associated with human infection: “top 6”. The procedures required a maximum of 6 hours for Reverse Dot Blot and 2 hours for Real Time PCR.

Statistical analys~~i~~es

Data are provided in absolute numbers and percent with 95% confidence intervals or as median and interquartile range (IQR) Correlation between variables was analyzed by means of the Pearson ~~'s~~ correlation coefficient. Chi squares test was used to compare categorical variables. The Student~~'s~~ t test was used to compare discrete variables. Statistical significance was set at a ~~p~~*P* value of < 0.05 (2-tailed). Data analysis was performed using StatView (Abacus Corp~~.~~, California, USA).

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Results

Bloody diarrhea

A total of 4767 patients with BD have been screened for the presence of *Stx* genes during the past 10 years. Male ~~gender~~ sex was significantly (p: 0.001) over-represented (56.9%) compared ~~to~~ with the expected 48.8% of the general population. The median age of screened patients was 3.4 years (IQR

1.5-7.0). BD was more common in younger children with the highest relative frequency in the age group <1 yr. More than 60% of screened patients were in the age range 0-5 years. Additionally, as known and expected, BD was more common during summer with a peak relative frequency in August and the nadir in February.

Shiga Toxin Gene Positivity

Out of the 4767 screened samples of BD, 214 (4.5%) were positive for either *Stx1* (n:62; 29.0%) or *Stx2* (n:97; 45.3%) or both genes (n:55; 25.7%). Moreover, 741 (15.5%) patients with BD were positive to the *intimin* (*eae*) gene either in association with *Stx* genes (n:124) or without (n: 617); in 85 samples (1.8%) the *invasion plasmin antigen H* gene (*ipaH*) was identified.

The rate of *Stx* gene positivity among children with BD per year ranged from as low as 2.5% in 2010 to 5.6% in 2016 with a positive slope of the regression line suggesting an increasing incidence of STEC infection over time (Figure 2).

During the same period of time, 95 children were also tested because of ongoing HUS associated with either BD (n:69) or non-BD (n:26) thus the total of *Stx* gene positive children identified during the 10 years, raises to 277.

Although the age group more commonly affected by bloody diarrhea is <1 year, this age-group seems relatively less affected by STEC infection compared ~~to~~ with other age groups. From age 1 yr onward, the percentage of *Stx*+ among BDs remains fairly constant across ages ranging from 7% to 9% thus demonstrating that no age is exempt from STEC infection. However, more than 50% of STEC infections were recorded below age 5 (Table ~~1~~I).

BD is more common in late summer and autumn, and the rate of *Stx* gene positivity among children with BD is higher from July through October. In September the rate of *Stx gene* positivity for BD observed, was well above 10% (almost three-fold the average).

Additionally no difference was observed in the ~~gender~~sex, age, or seasonal distribution of identified *Stx* genes (1, 2 or1+2).

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Among *Stx*+ patients captured by the screening program, the most frequently identified serotype was O157 (25.3% of cases), followed by O26 (24.9%), O103 (7.0%), O111 (6.1%), O145 (4.4%), O127 (1.3%) and O104 (0.4%). Non-top6 serotypes accounted for 30.6% of cases positive for *Stx* at the time of screening for BD.

STEC-HUS

Out of the 214 *Stx*+ patients identified through the screening program, only 34 developed HUS (15.9% of *Stx* + and 0.71% of screened BD). Only STEC-infected patients carrying either *Stx*2 gene (with or without *Stx*1) developed STEC-HUS. Thus, if the analysis is restricted to the 152 patients carrying *Stx*2 gene (either alone or in combination with *Stx*1) the subjects at actual risk of STEC-HUS was 3.2% of BDs (152/4767). The risk of *Stx* gene positive infection to turn into STEC-HUS was significantly different according to the isolated *Stx* gene: 0% for *Stx*1, 23.7% for *Stx*2 and 12.7% for *Stx*1+2 (p: 0.0001).

Out of 63 patients who were Stx2+ that were tested with urine dipsick or urinalysis for hemoglobinuria, all those with HUS, had a positive urine and only 7 positive testes were not associated with HUS. The median number of days between presentation and the detection of hemoglobinuria was 4.8 (IQR: 3.3-6.0) and whenever the urine turned positive for hemoglobinuria the diagnosis of HUS wasconfirmed by bloodtestswithin the subsequent 24 hours. None of the patients persistently negative for hemoglobinuria developed HUS. Thus, the sensitivity of hemoglobinuria for the development of HUS was 100% (95%CI 95-100%) with a specificity of 85% (95%CI 77-91%); the positive predictive value was 68% (95%CI 55-79), the negative predictive value was 100% (93-100) and the accuracy was 89% with a likely hood ratio of 6.7.

~~While~~ Although *Stx* gene positivity (as percent of BDs) does not change with age (ranging from 2.6 to 4.3), the risk of conversion of *Stx*+ into STEC-HUS decreased with age, being as high as 26.4% in children younger than 5 years old (P for trend: 0.10) (Table 1).

With regard to *Stx* genes involved in the 129 patients who developed HUS, Stx1 alone was found in 0.8% only, ~~while~~ and the most common association was with *Stx*2 and *Stx*1+2 (details are provided in Table 2). The most frequent STEC serotype identified among children with HUS was O26 (34.1%) ~~while~~ and O157 was detected in 17.8% of cases. The distribution of serotypes was significantly different in patients whose STEC infection who did or did not turn into HUS (Table 2).

The median creatinine level (and IQR) at presentation of STEC-HUS in children who were identified as STEC infected by the screening program prior to the development of the renal complication (n:34) was significantly (p:0.001) lower (0.9 mg/dL; IQR 0.4-1.5) compared ~~to~~ with that of patients diagnosed with ongoing STEC-HUS with BD (n:95; 1.5 mg/dL; IQR 0.9-2.9). As shown in Figure 1, no significant differences were observed in the rate of short-term complications (need for RRT or CNS involvement) between the 2 groups. The overall long-term outcome was more favorable in patients diagnosed with STEC infection prior to HUS development (any adverse long-term outcome: 2.9% vs 15.8%). Moreover, the distribution of Stx type and of serotypes were not different in patients whose STEC infection was identified before or following the diagnosis of HUS. Finally, out of 129 children with HUS managed at our Center during the 10 years of activity of the screening program, only one died (0.8%; 95%CI 0.02-4.3).

Discussion

BD is not uncommon in children and, when caused by STEC, it can be complicated by the development of STEC-HUS, with possible life-threatening consequences, including severe acute and chronic renal damage. ~~So far,~~ N~~n~~o specific treatment for STEC-HUS is available and the management of patients is centered on supportive care.

In 2010, we became increasingly aware that control of STEC-HUS required strong preventive measures. Thus, we decided to move our attention from overt HUS to the early (prodromal) phase of the infection when the kidney is not yet symptomatically involved. The target of our intervention was to decrease the incidence of STEC-HUS while ameliorating its course and outcome. This ambitious target was supported by the increasing availability of reliable diagnostic tools as well as by new evidence that well hydrated children exhibited better outcomes (20). The working hypothesis behind the activity of the ItalKid-HUS Network was that the screening of BDs for Stx could lead to the early identification, referral and inpatient management of STEC infected children at high risk for STEC-HUS.

~~Ten years after the beginning of this effort we present our finding.~~ Our efforts did not decrease the incidence of STEC-HUS, which has remained fairly stable in our area (around 5-6 cases/million age-related population). We also observed that the disease often has ~~such~~ a rapid course from the development of BD to STEC-HUS , thus~~that~~ only 1/3 of STEC-HUS were identified at the stage of BD prior to the development of the TMA (Figure 1). ~~Another critical issue is that~~ ~~m~~More than 20% of STEC-HUS cases did not have BD or BD was not noticed, so the screening did not capture them. Nevertheless, the program has provided evidence of a positive impact on the disease. First of all, we now have a much better understanding of the local epidemiology of the disease that leads to more detailed individual risk assessment with important clinical implications. For instance, we are now aware that 4.5% of all children with BD are Stx+. Furthermore, we confirmed that Stx 1 is a very rare cause of STEC-HUS ~~while~~ and Stx2 alone is associated with a higher (double) risk than when present in combination with Stx1 as previously hypothesized from both human (21-25) and experimental data (26-27). In addition, although BD is more common in very young children (<1 yr. old), these seem relatively less likely to be Stx+ compared ~~to~~ with other age groups.

Our screening program clearly identified only a portion of BDs occurring in the area and this is among the reasons why a substantial number of HUS cases were not captured before the development of the renal complications. Nevertheless, because of the screening program and the consequent awareness

among pediatricians of BD presenting as a prodromal phase of a subsequent severe disease, the diagnosis of STEC-HUS was significantly anticipated in patients who entered the screening program compared ~~to~~ with unscreened patients as well. For example, the median level of serum creatinine (sCr) at STEC-HUS presentation in the screened patients was 0.9 mg/dL compared with 1.5 mg/dL in unscreened patients. Furthermore, the sCr level during the years immediately prior to the initiation of the screening program of BD for Stx at our Center was 2.0 mg/dL (IQR 1.1-3.3) [20]. In addition, if we analyze some recently published series of STEC-HUS, the sCrof our patients at presentation is significantly lower: in Belgium, Keenswijk ~~W.~~ et al~~.~~ reported a median value of 2.98 mg/dL in 34 patients [28], in Argentina, Alconcher et al~~.~~ reported a median sCr at presentation of 2.39 mg/dL in 466 patients [29] and that reported by Balestracci et al~~.~~ was 2.35 mg/dL in 153 patients [30]. Finally, in a series from France, Netherlands and UK, altogether involving 270 patients, sCr at admission was well above 2 mg/dL (unpublished data kindly provided by Chantal Loirat, Veronique Fremeaux-Bacchi, Nicole van der Kar and Sally Johnson).

Finally, among the major results of anticipating the diagnosis and the management of STEC infections and of STEC-HUS, we observed an important drop of the case-fatality rate from 5.2% [27] to less than 0.8%. However, asshown in Figure 1, early diagnosis did not affect the acute phase of the disease (both in terms of need for RRT and overt CNS involvement) but early diagnosis did reduce the overall long-term sequelae of the disease. Given the relatively small numbers of BD in children, the severity of the possible complications and their rapid development, we believe that all patients with BD should be closely monitored and managed as if any of them could evolve into STEC-HUS until Stx testing excludes the diagnosis. In our setting, close monitoring and appropriate management means stool analysis for Stx, weight restoration (if dehydrated), generous maintenance fluid and urine dip-stick every 12 hours aimed at identifying upcoming TMA (Figure 3).

Only patients carrying Stx2 (alone or in combination with Stx1) (3.2% of BDs) will continue to require the described measures (generous fluid supplementation and urine dip stick every 12 hours) until diarrhea resolves. Almost 15% of this subgroup will develop STEC-HUS. This becomes

particularly relevant during late summer and early fall when the probability that BD is associated with Stx rises well above 10%.

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References

1. WHO Data - May 2 2017. https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease.
2. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull WHO 2003; 81: 197-204.
3. Meng CY, Smith BL, Bodhidatta L, Richard SA, Vansith K, Thy B, et al. Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. Pediatr Infect Dis J 2011; 30:331–335.
4. Qu M, Deng Y, Zhang X, Liu G, Huang Y, Lin C et. al. Etiology of acute diarrhea due to enteropathogenic bacteria in Beijing, China. J Infect 2012;65(3):214-22. Epub 2012 Apr 27.
5. Household KC, Mann MD, Bowie MD. Enteropathogens associated with acute infantile diarrhea in Cape Town. S Afr Med J 1988; 73: 83-87.
6. Presterl E, Zwick RH, Reichmann S, Aichelburg A, Winkler S, Kremsner P, et al. Frequency and virulence properties of diarrhea genic Escherichia coli in children with diarrhea in Gabon. Am J Trop Med Hyg 2003; 69: 406-410.
7. Saidi SM, Iijima Y, Sang WK, Mwangudza AK, OundoJO, TagaK,et. al. Epidemiological study on infectious diarrhea diseases in children in a coastal rural area of Kenya. Microbiol Immunol 1997; 41: 773-778.
8. Brooks JT, Shapiro RL, Kumar L, Wells JG, Phillips-Howard PA, Shi YP, et al. Epidemiology of sporadic bloody diarrhea in rural Western Kenya. Am J Trop Med Hyg 2003; 68: 671-7.
9. Rao MR, Abu-Elyazeed R, Salvarino SJ, Naficy AB, Wierzba TF, Abdel-Messih I, et. al. High disease burden of diarrhea due to Enterotoxigenic Escherichia coli among rural Egyptian infants and young children. J Clin Microbiol 2003; 41: 4862-4864.

10. Cho SH, Shin HH, Choi YH, Park MS, Lee BK. Enteric bacteria isolated from acute diarrheal patient in the Republic of Korea between the year 2004 and 2006. J Microbiol 2008;46(3):325-30.

11. Hilmarsdóttir I, Baldvinsdóttir GE, Harðardóttir H, Briem H, Sigurðsson SI. Enteropathogens in acute diarrhea: a general practice-based study in a Nordic country. Eur J Clin Microbiol Infect Dis 2012; 31:1501–1509.

12. Maraki S, Ladomenou F, Samonis G, Galanakis E. Long-term trends in the epidemiology and resistance of childhood bacterial enteropathogens in Crete. Eur J ClinMicrobiol Infect Dis 2012; 31:1889–1894.

13. Mota MI, Gadea MP, GonzálezS, González G, Pardo L, Sirok A, et al. Bacterial pathogens associated with bloody diarrhea in Uruguayan children. Revista Argentina de Microbiología 2010; 42: 114-117.

14. Sang W, Boga H, Waiyaki P, Schnabel D, Wamae N, Kariuki S, et al. Prevalence and genetic characteristics of Shigatoxigenic Escherichia coli from patients with diarrhoea in Maasailand, Kenya. J Infect Dev Ctries 2012; 6(2):102-108.

15. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis 1999; 5:607–625

16. Buzby JC, Roberts T, Jordan Lin C-T, MacDonald JM. Bacterial foodborne disease: medical costs and productivity losses. Economic Research Service, U.S. Department of Agriculture. Agricultural Economics Report No. AER741 1996; Available online at: http://www.ers.usda.gov/Publications/AER741/. Accessed 8 June 2011.

17. Holtz LR, Neill MA, Tarr PI. Acute Bloody Diarrhea: A Medical Emergency for Patients of All Ages. Gastroenterology 2009; 136(6):1887-98.

18. Ardissino G, Salardi S, Colombo E, Testa S, Borsa-Ghiringhelli N, Paglialonga F, et al. Epidemiology of haemolytic uremic syndrome in children. Data from the North Italian HUS network. Eur J Pediatr 2016;175(4):465-73.

19. EU-RL VTEC (European Union Reference Laboratory VTEC, Identification and characterization of Vercytotoxic-producing Eschericha Coli (VTEC) by real Time PCR amplification of the main virulence geneciated with the serogroups mainly associated with severe human infections EU-RL VTEC. Method-02-Rev0. http://old.iss.it/vtec/index.php?lang=2&anno=2020&tipo=3.
20. Ardissino G, Tel F, Possenti I, Testa S, Consonni D, Paglialonga F, et al. Early Volume Expansion and outcomes inhemolytic uremic syndrome. Pediatrics2016;137(1).
21. Tarr G, Stokowski T, Shringi S, Tarr P, Freedman S, Oltean H, et al. Contribution and Interaction of Shiga Toxin Genes to Escherichia coli O157:H7Virulence. Toxins 2019; 11: 607.
22. Persson S, Olsen KE, Ethelberg S, Scheutz F. Subtyping method for Escherichia coli shiga toxin (verocytotoxin) 2 variants and correlations to clinical manifestations. J ClinMicrobiol2007; 45:2020-2024.
23. Luna-Gierke RE, Griffin PM, Gould LH, Herman K, Bopp CA, Strockbine N, et al. Outbreaks of non-O157 Shiga toxin-producing Escherichia coli infection: USA. EpidemiolInfect 2014; 142:2270-2280.
24. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in Escherichia coli O157:H7 infections. J Infect Dis 1989; 160:994-998
25. Jelacic S, Wobbe CL, Boster DR, Ciol MA, Watkins SL, Tarr PI, et al. ABO and P1 blood group antigen expression and stx genotype and outcome of childhood Escherichia coli O157:H7 infections. J Infect Dis2002; 185:214-219.
26. Russo LM, Melton-Celsa AR, O'Brien AD. Shiga Toxin (Stx) Type 1a Reduces the Oral Toxicity of Stx Type 2a. J InfectDis 2016; 213(8):1271-1279.

27. Donohue-Rolfe A, Kondova I, Oswald S, Hutto D, Tzipori S. Escherichia coli O157:H7 strains that express Shiga toxin (Stx) 2 alone are more neurotropic for gnotobiotic piglets than are isotypes producing only Stx1 or both Stx1 and Stx2. J InfectDis 2000;181(5):1825-1829.

28. Keenswijk W, Vanmassenhove J, Raes A, Dhont E, VandeWalle J. Blood urea nitrogen to serum creatinine ratio is an accurate predictor of outcome in diarrhea-associated hemolytic uremic syndrome, a preliminary study. European Journal of Pediatrics 2017; 176; 355–360

29. Balestracci A, Mariel SM, ToledoI,Alvarado C, Wainsztein RE. Laboratory predictors of acute dialysis in hemolytic uremic syndrome. Pediatrics International 2014; 56, 234–239

30. Alconcher LF, Coccia PA, Suarez ADC, Monteverde ML, Perez Y Gutiérrez MG, Carlopio PM, et al. Hyponatremia: a new predictor of mortality in patients with Shiga toxin-producing Escherichia coli hemolytic uremic syndrome. PediatrNephrol2018;33(10):1791-1798.

Figures and Tables

Figure 1: Schematic representation of the results of the ItalKid-HUS Network activity (2010-2019): screened bloody diarrheas for Stx, diagnosed HUS by screening group and related laboratory at HUS diagnosis.

Legend: STEC-HUS: shiga toxin-relate hemolytic uremic syndrome, Stx: Shiga Toxin, sCr: serum creatinine, IQR: interquartile range; PTL: platelets count; Hb: hemoglobin; RRT: renal replacement therapy, CNS: central nervous system, CKD: chronic kidney disease, §: IDDM (n: 2) and radial artery thrombosis with consequent finger amputation (n: 1),*: $p<0.001$ vs other groups.

Figure 2: Changes in the rate of Stx2- and Stx1+2-positive bloody diarrheas over time in the area of the ItalKid-HUS Network (figures include all patients identified positive including patients with ongoing HUS referred to our Center during the analyzed period: screened and unscreened).

Figure 3: Diagnostic management of bloody diarrhea focused on the risk of STEC-HUS used in the ItalKid-HUS Network.

Legends: Stx: Shiga Toxin, uHb: hemoglobinuria; PTL: platelets.

Table 1 – Distribution of bloody diarrhea, of Stx 2 and Stx1+2 positivity and of eHUS by age groups including only patients being tested at the stage of bloody diarrhea (prior to the development of eHUS).

Table 2 – Virulence profile and serotypes in patients infected with STEC and in those who developed STEC-HUS (the table includes all patients: screened and unscreened).

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Data Sharing Statement

Deidentified individual participant data will not be made available.

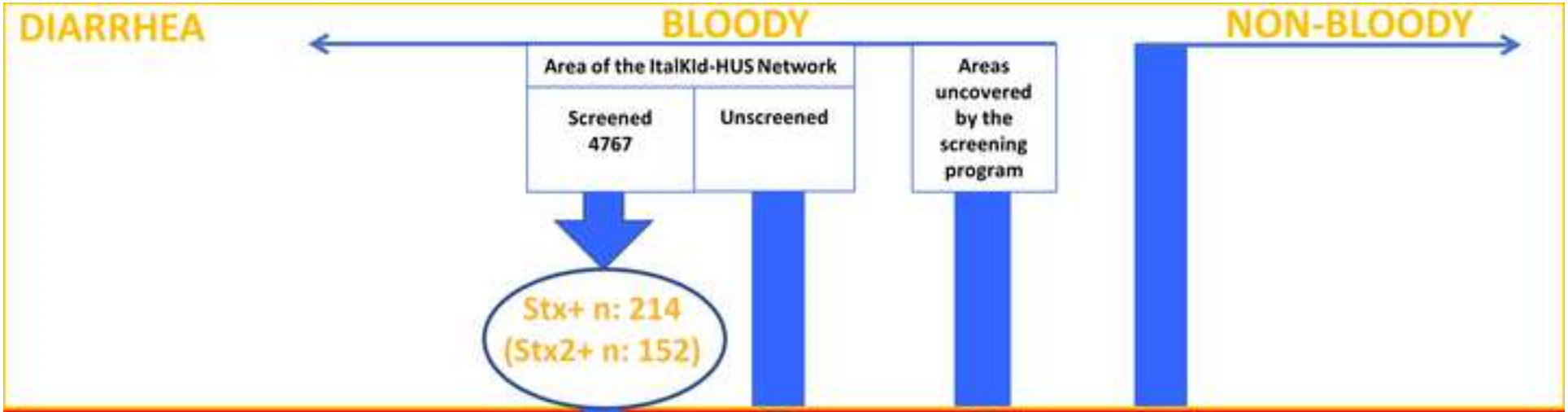
Table 1.

Age group (yr)	Bloody Diarrhea (%)	Stx2 & 1+2 Pos. (% of BD)	STEC-HUS (% of Stx Pos.)
0-5	3078 (64.6)	87 (2.8)	23 (26.4)
5-10	1012 (21.2)	40 (4.0)	8 (20.0)
10-15	491 (10.3)	21 (4.3)	3 (14.3)
15-20	186 (3.9)	4 (2.6)	0 (0.0)
Total	4767 (100)	152 (3.2)	34 (22.4)

Table 2.

	STEC+ w/o HUS (n. 180)	**STEC-HUS (n. 129)**	**Chi-square**
Stx, n (%) 1 2 1+2 unknown	 61 (33.9) 72 (40.0) 47 (26.1) NA	 1 (0.8) 71 (55.0) 21 (16.3) 36 (27.9)	p<0.001
Eae+, n (%)	124 (68.9)	78 (60.5)	p=0.12
Serotype, n (%) O157 O26 Other top6 Non top6 Unknown	 35 (19.4) 15 (8.3) 22 (12.2) 42 (23.3) 66 (36.8)	 23 (17.8) 44 (34.1) 22 (17.1) 28 (21.7) 12 (9.3)	p<0.001

Figure 1



			P
Laboratory at HUS diagnosis			
sCr, mg/dL, median (IQR)	0.9 (0.4-1.5)	1.5 (0.9-2.9)	0.001
PTL, x10^3 /mm^3, median (IQR)	69 (41-97)	61 (37-93)	0.327
Hb, g/dL, median (IQR)	10.1 (9.0-11.8)	9.5 (7.8-11.1)	0.076
Short-term complications			
Need of RRT, n (%)	17 (50)	32 (34)	0.093
CNS Involv, n (%)	3 (8.8)	11 (12)	0.658
Long-term adverse outcome			
CKD, n (%)	1 (2.9)	12 (12.8)	0.096
Extrarenal sequels[§], n (%)	0 (0.0)	3 (3.2)	0.309
Death, n (%)	0 (0.0)	1 (1.5)	0.546
Any adverse long-term outcome	1 (2.9)	15 (15.8)	0.051

Figure 2

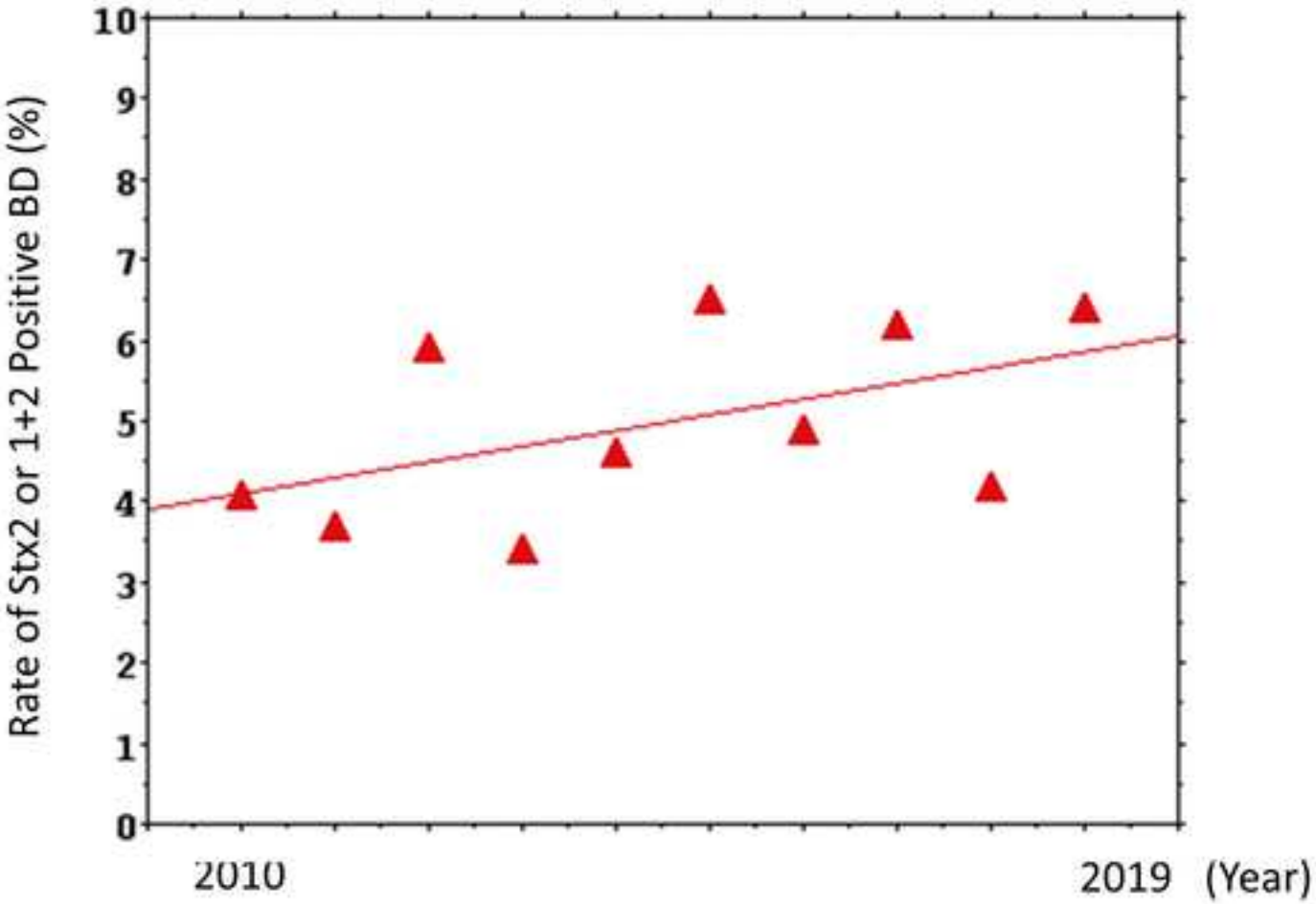


Figure 3

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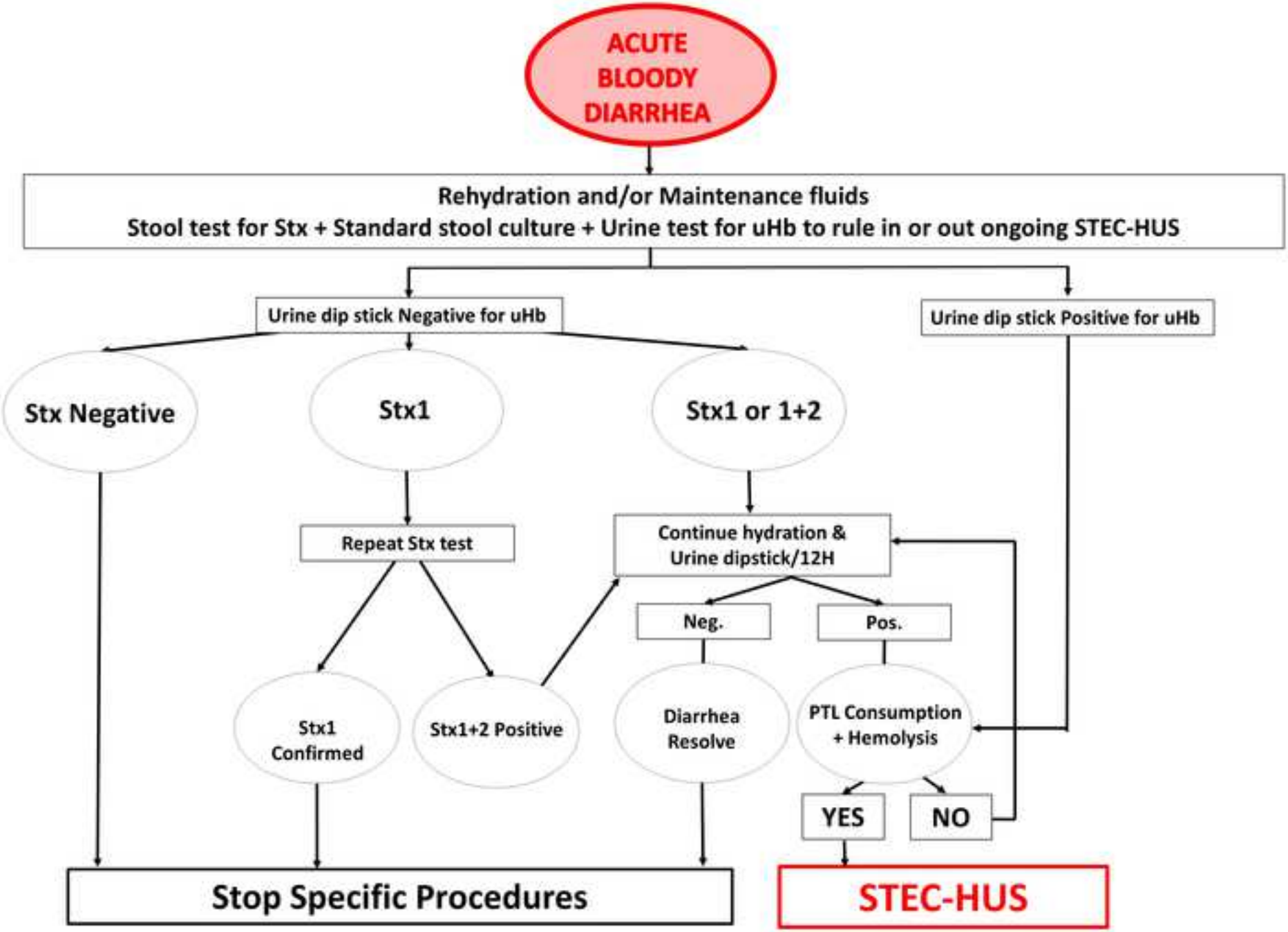

Appendix

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Data in Brief

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