Postinfectious Hemolytic Uremic Syndrome

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Abbreviations

A/E	Attaching and effacing
ACE(i)	Angiotensin converting enzyme (inhibitor)
ADAMTS13	A disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13
ADC	Apparent diffusion coefficient
aHUS	Atypical hemolytic uremic syndrome
AIDS	Acquired immunodeficiency syndrome
AKI	Acute kidney injury
ALT	Alanine amino transferase
AP	Alternative pathway (of complement)
ARDS	Acute respiratory distress syndrome
Bcl-2	B-cell lymphoma protein-2
BP	Blood pressure
C1q	Complement factor 1q
C3	Complement factor 3
C4	Complement factor 4
C5	Complement factor 5
CBC	Complete blood cell (count)
CDC	Centers for Disease Control and Prevention
CFB	Complement factor B
CFH	Complement Factor H
CFHL-1	Complement factor H-like 1
CFHR-1	Complement factor H-related protein 1
CI	Confidence interval
CKD	Chronic kidney disease
CNS	Central nervous system
CPKDRC	Canadian Pediatric Kidney Disease Research Centre
CrI	Credible interval
CRP	C-reactive protein
CRRT	Continuous renal replacement therapy
DAF	Decay-accelerating factor
DGKE	Diacylglycerol kinase-epsilon
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
DWI	Diffusion-weighted images
EAEC	Enteroaggregative *E. coli*
eGFR	Estimated glomerular filtration rate

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D.F. Geary, F. Schaefer (eds.), *Pediatric Kidney Disease*, DOI 10.1007/978-3-662-52972-0_26

EHEC	Enterohemorrhagic *Escherichia coli*
eHUS	Enteropathogen (or *Escherichia coli*) induced hemolytic uremic syndrome
EhxA	Enterohemolysin
ELISA	Enzyme-linked immunosorbent assay
EPEC	Enteropathogenic *Escherichia coli*
ER	Endoplasmic reticulum
ESA	Erythropoiesis-stimulating agent
Esp	Extracellular serine proteases
ESRD	End stage renal disease
FDA	Federal Drug Agency
FLAIR	Fluid-attenuated inversion recovery
Gb3	Globotriaosylceramide
Gb4	Globotetraosylceramide
GI	Gastrointestinal
Hb	Hemoglobin
HC	Hemorrhagic colitis
HD	Hemodialysis
HLA	Human leukocyte antigen
HUS	Hemolytic uremic syndrome
IA	Immunoabsorption
Iha	Iron-regulated gene A (IrgA) homolog adhesin
iHUS	Influenza-induced hemolytic uremic syndrome
IPD	Invasive pneumococcal disease
IQR	Interquartile range
IrgA	Iron-regulated gene A
IV	Intravenous
JNK	c-Jun N-terminal kinase
KatP	Catalase/peroxidase
LDH	Lactate dehydrogenase
LEE	Locus of enterocyte effacement
LPS	Lipopolysaccharide
MAHA	Microangiopathic hemolytic anemia
MAP(K)	Mitogen-activated protein (kinase)
MCP	Membrane cofactor protein
MIC	Minimal inhibitory concentration
MMACHC	Methylmalonic aciduria and homocystinuria, cblC type (gene)
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NA	Neuraminidase
NanA	Neuraminidase A
NM	Non-motile
NSAID(s)	Non-steroidal anti-inflammatory drug(s)
NYED	Not yet etiologically defined
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor type 1
PCR	Polymerase chain reaction
PD	Peritoneal dialysis
PE	Plasma exchange
PEITC	Phenethyl isothiocyanate
PI	Plasma infusion
pnHUS	Pneumococcal (*Streptococcus pneumoniae*) hemolytic uremic syndrome
PO	Per oral
PRBC	Packed red blood cell(s)
PRES	Posterior reversible leukoencephalopathy syndrome
PSGL-1	P-selectin soluble ligand 1
PT	Prothrombin time
PTT	Partial thromboplastin time
RAS	Renin-angiotensin system
RBC	Red blood cell(s)
RNA	Ribonucleic acid
RRT	Renal replacement therapy
rTM	Recombinant thrombomodulin
Saa	STEC autoagglutinating adhesin
SC5b-9	Serum (soluble complement factor) C5b to 9 complex (see TCC)
SCR	Short consensus repeat(s)
sCR1	Soluble complement receptor 1
SD1	*Shigella dysenteriae* type 1
SLT	Shiga-like toxin
SMAC	Sorbitol MacConkey (agar)
SMX/TMP	Sulfamethoxazole/trimethoprim
STEC	Shiga toxin producing *Escherichia coli*
STPB	Shiga toxin producing bacteria
Stx	Shiga toxin
SubA	Subtilase A
T3SS	Type III secretion system
TCC	Terminal complement complex
TF	Thomsen-Friedenreich (antigen)
THBD	Thrombomodulin (gene)
Tir	Translocated intimin receptor

TM	Thrombomodulin
TMA	Thrombotic microangiopathy
TNF-α	Tumor necrosis factor alpha
TTP	Thrombotic thrombocytopenic purpura
UK	United Kingdom
US	United States (of America)
USS	Upshaw Shulman syndrome
UTI	Urinary tract infection
VEGF	Vascular endothelial growth factor
VT	Vero(cyto)toxin
VTEC	Vero(cyto)toxin producing *Escherichia coli*
WBC	White blood cell

Introduction

For the purpose of this section, we define postinfectious hemolytic uremic syndrome as HUS [1] caused by specific infectious organisms in patients with no identifiable HUS-associated genetic mutation or autoantibody. Major triggers of postinfectious HUS are Shiga toxin (Stx) producing bacteria (STPB, mainly *Escherichia coli* and *Shigella dysenteriae* type 1) [2, 3] and neuraminidase (NA) producing organisms (mainly *Streptococcus pneumoniae*) [4, 5]. Stx and NA are thought to injure vascular endothelial and perhaps circulating red blood cells and platelets leading to thrombotic microangiopathy with intravascular hemolysis (TMA). In children, more than 80 % of cases of HUS are due to STEC infection. This contrasts with HUS causally linked to the deficiency of proteins that regulate the alternative pathway of complement, either due to genetic mutations or the presence of autoantibodies (mainly to complement factor H), or to other genetic and metabolic causes (Box 26.1). However, HUS can arise following infection by a "specific" agent in a patient with a complement defect; the "atypical" nature of such HUS is usually uncovered by its atypical presentation (relapsing course, recurrence after transplantation or family history).

Box 26.1 Classification of HUS/TMA and TTP

1. Infection-induced HUS (caused by endothelial injury due to specific infectious agents)
   - (a) Shiga toxin-producing bacteria (STPB)
     - (i) Shiga toxin-producing / enterohemorrhagic *Escherichia coli* (STEC/EHEC)
     - (ii) *Shigella dysenteriae* type 1
     - (iii) *Citrobacter freundii* and others
   - (b) Neuraminidase-producing bacteria
     - (i) *Streptococcus pneumoniae*
     - (ii) *Clostridium perfringens* and others
   - (c) Influenza A virus (A/H3N2, A/H1N1)[a]
   - (d) Human immunodeficiency virus (HIV)[b]
2. Hereditary/genetic forms of HUS
   - (a) HUS associated with mutations of regulatory proteins and components of the complement and coagulation pathways[c]
     - (i) Soluble regulator deficiencies (examples: CFH, CFI etc.)
     - (ii) Membrane-bound regulator deficiencies (examples: MCP)
     - (iii) Thrombomodulin, plasminogen[d]
   - (b) Genetic abnormalities without known complement dysregulation, usually autosomal recessive (examples: defective cobalamin metabolism due to mutations in *MMACHC* [methylmalonic aciduria and homocystinuria, cblC type]; mutation of *DGKE* [diacylglycerol kinase-epsilon])[e]
3. Autoimmune HUS
   - (a) Autoantibodies against complement regulatory proteins (example: anti-CFH antibody)
4. Thrombotic thrombocytopenic purpura (TTP)
   - (a) Hereditary TTP (Upshaw Shulman Syndrome [USS], autosomal recessive mutation of *ADAMS13*)
   - (b) Autoimmune TTP (due to anti-ADAMS13 antibody)
5. NYED (not yet etiologically defined)
   - (a) Spontaneous forms without known co-morbidities
   - (b) "Secondary forms" (examples: HUS associated with bone marrow transplantation,[f] anti-phospholipid syndrome, malignant hypertension etc.

	(c) HUS caused by endotheliotoxic therapeutics (examples: cancer drugs, endotheliotropic antibodies, such as anti-VEGF)

[a]While influenza virus expresses neuraminidase (NA), its causal role in HUS has yet to be proven
[b]The mechanism underlying HIV HUS is not clear; the majority of patients appear to present HUS-like features [6–8]
[c]Forms involving unregulated alternative pathway of complement activation are differentiated from others for therapeutic purposes; combinations of various factor mutations and/or autoantibodies exist
[d]For details, see [9]
[e]A recent publication implicates cases with DGKE mutation that demonstrate complement consumption [10]
[f]Combination with genetic complement regulator mutations have been described [11]

The historical terms diarrhea-positive (D^+) and diarrhea-negative (D^-) HUS, introduced to distinguish STEC-induced HUS from "atypical" forms, should be abandoned since at least one third of patients with complement-mediated "atypical" HUS present with diarrhea or even colitis [12]. The D^+/D^- dichotomy fails to differentiate postinfectious forms, such as *S. pneumonia* HUS, from "atypical" HUS, and could delay the necessary workup and potentially deprive patients of effective treatment. An etiology-based classification is preferred.

Finally, there is emerging evidence of (transient) complement activation in post-infectious forms of HUS in the absence of a demonstrable genetic defect or anti-CFH autoantibodies. The precise mechanism of complement activation and its pathological significance are presently under investigation. With the evolving understanding of the complement system and of the pathogenesis of different forms of HUS, some of the descriptions and assumptions in this chapter will have to be revised in the future [12–15].

Shigatoxin Producing *Escherichia coli*-HUS

History of HUS and Definitions

The term "hemolytic uremic syndromes" was first used in 1955 by the Swiss hematologist Dr. C. Gasser, who described five children presenting with the triad of acute hemolytic anemia, thrombocytopenia and renal failure [1]. The largest early series of patients with HUS originated from Argentina, the country with the highest incidence of HUS [16].

It was not until 1983 when two major discoveries led to the recognition of a specific microbial etiology as the predominant cause of HUS in children: Dr. Karmali and his group from the Hospital for Sick Children in Toronto, Canada reported the isolation of *Escherichia coli* strains from children with "idiopathic" (typical) HUS that produced a filterable agent that was toxic to cultured Vero (African Green Monkey kidney) cells (verocytotoxin or VT) [2, 17]. VT detected in the stools of children with HUS (free fecal VT) and lysates from VT producing *E. coli* (VTEC) [18] were neutralized by convalescent patient and rabbit immune sera [2, 17]. In the same year, Dr. Allison O'Brien from the Armed Services, Bethesda, recognized that a toxin, elaborated by a newly described *E. coli* strain (*E. coli* O157:H7), bore close similarity with the toxin produced by *Shigella dysenteriae* type 1 (Shiga toxin) which she termed Shiga-like toxin (SLT) [19]. The prototypic disease caused by *E. coli* O157:H7 became known as hemorrhagic colitis [20–23] and the organism was termed "enterohemorrhagic *E. coli*" or EHEC. Subsequent work by Karmali, O'Brien, Karch and others showed that verotoxin, SLT and the classic Shiga toxin belonged to a family of closely-related bacterial protein exotoxins with the major subdivisions of SLT-I or VT1, now termed Stx1, and SLT-II or VT2, now termed Stx2 (and variants) [24] as outlined in Table 26.1. We will use the terms STEC-HUS, Stx-HUS or enteropathogenic HUS (eHUS) to describe patients with the "typical" or "classical" form of HUS.

Table 26.1 Shiga toxins: nomenclature, reservoir and clinical relevance

Stx family	Current nomenclature[a]	Type strain	Stx synonyms	Sequence homology[b]		Clinical association	References
Stx types	*Stx subtypes*			*A subunit*	*B subunit*		
Stx 1	Stx1	*S. dysenteriae* type 1 (SD1)	Stx	100 %	100 %	Dysentery, HUS	[25, 26]
	Stx1a	*E. coli* O157:H7	STLI, VT1	99 %	100 %	Hemorrhagic colitis (HC), HUS	[27–29]
	Stx1c	*E. coli* O128:H2	SLTIc, VT1c	97 %	97 %	Uncomplicated diarrhea/ asymptomatic humans, ovine, deer	[30, 31]
Stx 2	Stx2a	*E. coli* O157:H7	SLTII, VT2, Stx2	100 % (homology to Stx 55 %)	100 % (homology to Stx 57 %)	HC, diarrhea, HUS	[25, 32, 33]
	Stx2b	*E. coli* O118:H12	SLTIIb, VT2b, [VT2d]	94 %	89 %	Low pathogenicity in humans	[34]
	Stx2c	*E. coli* O157:H7	SLTIIc, VT2c	100 %	97 %	HC, HUS; often expressed jointly with Stx2a	[35, 36]
	Stx2d ($Stx2d_{activatable}$)	*E. coli* O91:H21	SLTIId, VT2d	99 %	97 %	HC, HUS	[34, 37]
	Stx2e	*E. coli* O139	SLTIIe, VT2e	94 %	87 %	Porcine edema disease; rare human diarrhea or HUS; binds to Gb3 and Gb4)	[38, 39]

Nomenclature base on References [24, 40]

[b]Additional Shiga toxins variants, produced primarily by non-human *E. coli* strains are Stx1d (ONT:H19, a bovine isolate), recognized by commercial ELISA, but not by prototypic anti-Stx1 mAb 13C4 [41], Stx2f (*E. coli* 128:H2, isolated from pigeon droppings) [42], and Stx2g (*E. coli* O2:H25, bovine isolate) [43]

[b]DNA sequence homology to prototypic Shiga toxin (Stx from *S. dysenteriae* 1) and Stx2a (STEC O157:H7 EDL)

There is some confusion about the designation of *E. coli* strains associated with hemorrhagic colitis (HC) and HUS. More than 200 serotypes have been described carrying Stx phage(s) and producing Stx, but only a limited number has been associated with human diseases [44]. The term STEC (Stx producing *E. coli*) describes *E. coli* strains harboring one or more Stx phages and producing Stx *in vivo*. EHEC (enterohemorrhagic *E. coli*) are defined by the disease they induce in humans, bloody (hemorrhagic) colitis [45]. STPB (Shiga toxin producing bacteria) encompass STEC and *S. dysenteriae* type 1 (SD1), and the occasional *Citrobacter freundii* [46–49], *Salmonella* or *Shigella* (enterobacteriaceae) isolates capable of Stx production [50].

While *E. coli* O157:H7 is worldwide the most important STPB and responsible for the majority of sporadic HC and HUS cases and outbreaks, non-O157:H7 STEC serotypes, including *E. coli* O111:H11/NM and O26H11/NM have been likewise implicated in (severe) human disease [49–61]. The large-scale *E. coli* O104:H4 outbreak in Germany in 2011 [62, 63] with >850 mostly adult victims of HUS and 50 deaths [64] represents a unique scenario where an enteroaggregative *E. coli* (EAEC) incorporated an *stx2* phage; this novel strain was propagated in a bean-sprouting facility and contaminated a product (sprouts), that is usually eaten raw [63, 65].

Epidemiology of STEC Infections and STEC HUS

Global estimates of the disease burden by human STEC infections and deaths did not exist until recently. Majowicz et al. recently published a study assessing the annual number of illnesses worldwide due to pathogenic STEC and the resulting cases of HUS, end-stage renal disease (ESRD) and death [66] using various online resources, including databases from 21 countries and WHO regions. According to the authors' (conservative) accounts, STEC causes about 2.8 million acute illnesses annually (95 % credible interval [$CrI_{95\%}$]: 1.7; 5.2 million), and leads to 3890 cases of HUS ($CrI_{95\%}$ 2400; 6700), 270 cases of ESRD ($CrI_{95\%}$ 20; 800), and 230 deaths ($CrI_{95\%}$ 130; 420).

One of the earliest and most comprehensive epidemiological studies of STEC infection and HUS was launched by the Canadian Pediatric Kidney Disease Research Centre (CPKDRC) in Ottawa following the discovery by Karmali and his group in Toronto, with the collaborative efforts of many Canadian centers [67]. The study revealed an annual incidence of sporadic (STEC) HUS in children younger than 15 years of 1.44 per 100,000. The vast majority of patients was diagnosed between the months April and September (82 % of cases); 72 % of patients were <5 years of age (median age 2.7 years). Diarrhea was present in 95 %; it was bloody in 74 % of patients. STEC O157:H7 was isolated in 51 % of those screened for this organism. Dialysis was performed in 48 %, and the mortality rate of this cohort was 2.7 % [67, 68].

During a 3-year, prospective CPKDRC study aimed at determining the risk of developing HUS after sporadic *E. coli* O157:H7 infection among 19 pediatric centers between 1991 and 1994, 582 children were identified with uncomplicated STEC gastroenteritis, 18 with isolated hemolytic anemia/partial HUS, and 205 with HUS (77 % with evidence of STEC infection) [69]. A complete cohort was available for Alberta, the Canadian province with the highest incidence of STEC infections. The risk of HUS after *E. coli* O157:H7 infection in Alberta was 8.1 % (95 % confidence interval, 5.3–11.6). The highest age-specific risk of HUS or hemolytic anemia was 12.9 % in young children <5 years of age [69]. This contrasted with a reported HUS risk of 31.4 % in participating tertiary care centers outside Alberta most likely reflecting referral bias; it highlights the need to critically read epidemiological studies in this field. In another CPKDRC study, 34 consecutive children with HUS were enrolled at 8 hospitals over a 4-month period; 16 patients were treated with dialysis (47 %), and 1 patient died (2.9 %). STEC O157:H7 was isolated from 26 patients, non-O157 from 4 patients, and 4 patients had no growth of STEC [68].

Additional case series, cohort studies and prospective, matched case-control, registry and

comparative studies have uncovered important epidemiological aspects of STEC infections and HUS. Detailed microbiological and molecular analyses of isolates obtained during outbreaks have identified the genetic signatures of pathogenic clones, ecological features and modes of transmission, and clinical phenotypes caused by the organism, mainly diarrhea, HC and HUS [52, 57, 58, 70–94].

STEC infections and STEC-HUS show a marked seasonal variation with a peak during the summer and early fall and only few cases during the winter of the Northern and Southern hemispheres. STEC infections and HUS are endemic in moderate climate zones and in areas of high-density cattle raising, such as Argentina, the pacific Northwest of the United States or Alberta in Canada, and Scotland, among others [95, 96]. Studies from Ontario, Canada showed that the prevalence of anti-Stx antibodies was greater in children from rural compared to urban areas suggesting earlier or more frequent exposure to STPB in the rural population. These and other surveys further suggest that the development of immunity to the toxin and/or the organism confers protection against Stx-mediated complications [97].

The majority of STEC-HUS cases appear to occur spontaneously. However, family members or close contacts often report a history of recent diarrhea. These endemic or "spontaneous" cases of HUS are epidemiological markers for the prevalence and (epidemic) transmission of STEC in the community or region. Its primary reservoir is cattle and cattle manure that contaminate produce and drinking water. STEC O157:H7 can survive for months or years and multiply at low rates even under adverse conditions [98].

The first etiologically defined epidemics of STEC infections and (fatal cases) of HUS in the early 1990s were linked to the consumption of contaminated ground beef [70, 72]. The outbreaks led to widespread media attention and expensive lawsuits, and eventually resulted in improved hygiene in slaughterhouses and warnings against the consumption of undercooked meat. A comprehensive review of 350 outbreaks of STEC O157:H7 infections in the United States between 1982 and 2002 [99] identified 8598 cases; 17 % of the patients were hospitalized, 4 % developed HUS, and 0.5 % died. The transmission was foodborne in 52 %, person-to-person in 14 %, waterborne in 9 %, and direct animal contact in 3 %. No vehicle was identified in 21 %. Of the foodborne outbreaks, 41 % were due to ground beef, and 21 % due to produce [99]. The role of processed meat as the predominant outbreak vehicle is diminishing, and more recent, large epidemics were due to contaminated well water [100] or agricultural produce, such as bean sprout (Sakai outbreak) [101, 102], lettuce [103] and fenugreek [63, 104, 105].

Non-O157:H7 STEC

While *E. coli* O157:H7 has been associated with the majority of outbreaks and of sporadic cases of STEC infections in many countries in North and South America, Central and Northern Europe, and China, non-O157:H7 STEC serotypes are increasingly recognized as a cause of sporadic as well as epidemic colitis and HUS. They belong to more than 50 *E. coli* serogroups [106–109].

Isolation frequencies of non-O157:H7 STEC strains approach or exceed those of O157:H7 strains in North America, Europe and elsewhere [55, 110, 111]. A study from the Centers for Disease Control and Prevention (CDC) in Atlanta, published in 2014, summarized outbreaks by non-O157 STEC infections in the US [109]. The authors defined "outbreak" as ≥2 epidemiologically linked, culture-confirmed non-O157 STEC infections. They reported 46 outbreaks with 1727 illnesses and 144 hospitalizations. Of 38 single-etiology outbreaks, two-third were caused by STEC O111:NM or O111:H8 (n = 14), and O26:NM or O26:H8 (n = 11); 84 % were transmitted in about equal proportions through food and person-to-person spread. Food vehicles included dairy products, produce (mainly fruits and vegetables) and meats, while the most common setting for person-to-person spread was childcare centers. About one-third of all STEC isolates were recovered in multiple-etiology outbreaks [108].

Severe hemorrhagic colitis is typically seen in infections by (enterohemorrhagic) *E. coli*

O157:H7 and O26:NM/H11 and less often with other STEC serotypes [65, 112, 113]. Per definition, all STEC strains have the potential to produce Stx. However, STEC serotypes and clones variably express additional pathogenic factors that mediate bacterial adherence in the gut and *in vivo* toxin production and delivery [114]. Deadly outbreaks by other non-O157 STEC strains have been reported, and the large *E. coli* O104:H4 epidemic in Northern Germany in 2011 was a powerful reminder that non-O157:H7 Stx producing *E. coli*, when introduced into widely distributed food items, can have devastating consequences.

HUS Risk

The HUS risk related to STEC infection varies substantially between STEC serotypes: in pediatric populations, it is between 8 % and 15 % for O157:H7 [69, 115]; it is generally lower, albeit less well defined, for most non-O157:H7 serotypes [109, 116]. A study from Germany estimated that *E. coli* O157:H7 imparts overall an approximately tenfold greater HUS risk compared with non-O157 STEC strains (<1 % versus 8–15 %) [116]. Nevertheless, there is substantial variation in the HUS risk according to the infecting (non-O157) STEC clone. In the cited US-based, CDC analysis [109] a greater percentage of persons infected by Stx2-positive non-O157:H7 STEC developed HUS compared with persons infected by Stx1-only producing strains (7 % vs. 0.8 %; $P<0{\cdot}001$) [109]. Infections by the emerging, highly pathogenic, Stx2-producing *E. coli* O26:NM/H11 [93, 117, 118] and, in particular, by the sorbitol-fermenting (SF) non-motile *E. coli* O157 clone [119], carry high case fatality rates between 11 % and 50 % [88, 120]. No animal reservoir has been identified for the "German" *E. coli* SF O157:NM strain, which has also been isolated from HUS patients in the Czech Republic and Finland [88, 121, 122].

Table 26.2 summarizes large or clinically significant outbreaks of STEC infections highlighting the spectrum of involved STEC serotypes and toxins, the vehicle of transmission and the calculated HUS risks.

Public Health Initiatives and Tools for Monitoring STEC Infections and Outbreaks

Since the recognition of the public health importance of STEC, national and international surveillance networks have been created with the goal to capture incipient outbreaks by comparing isolates from different laboratories. These networks are often linked to specialized, national or international reference laboratories using the full spectrum of serological, biochemical and advanced molecular typing technologies. Examples of surveillance networks are the CDC-sponsored FoodNet (http://www.cdc.gov/foodnet/) consisting of a network of pediatric nephrologists and hospital infection control personnel that catches 15 % of the US population with sites in ten states [142, 143], the Food- and Waterborne Diseases and Zoonoses Network (FSW-Net), FoodNet Canada [144], Eurosurveillance of the European Center for Disease Prevention and Control (ECDC) in Stockholm, Sweden (www.ecdc.europa.eu/), the "Institut de veille sanitaire" in France [145], and the OzFoodNet network in Australia (www.ozfoodnet.gov.au/) and others worldwide. These networks and their on-line and print publications are valuable sources of up-to-date trends and outbreak information.

Pathogenesis of STEC Disease and HUS

STEC are among the most dreaded enteric pathogens in moderate climates of the Northern and Southern hemispheres due to their potential to cause severe colitis and HUS. They display a sophisticated machinery involving bacterial and host proteins, high contagiosity and resistance to environmental factors. The central pathogenic factor leading to HC and HUS is the ability to produce Stx and to deliver the toxin into the circulation.

STEC are not tissue invasive, and bacteremia is not a feature of STEC diarrhea or HUS. The vascular (endothelial) injury and clinical and pathological changes are believed to result from

Table 26.2 STEC-HUS outbreaks and epidemics

Location (year)	Vehicle	Outbreak strain (Stx type)	# of cases (hospitalization)	# of HUS	HUS risk ratio	# deaths/HUS mortality	Ref
Upper Bavaria, (Germany) [Sept–Nov 1988]	?	*E. coli* O157:NM[b] (*stx2*)	6	6 (4–17 months; dialysis 6)	100 %	0/6	[123]
Lombardia, Italy [Apr–May 1992]	?	*E. coli* O111:NM (*stx1* and *stx2*)	?	9	?	1/9 (11.1 %)	[124]
State of Washington/ West Coast (USA) [Jan–Feb 1993]	Beef patties (Hamburger; errors in meat processing and cooking)	*E. coli* O157:H7 (*stx1* and *stx2*)	501 (151; 31 %) Children 278	45 (37 children)	9.0 % (Children 13.3 %)	3/45 (6.7 %) 3/501 (0.60 %)	[72] See also [125–128]
South Australia [Jan–Feb 1995]	Dry fermented sausage (Mettwurst)	*E. coli* O111:NM (*stx1* and *stx2*)	?	21	?	1/21 (4.8 %)	[75, 76]
Sakai, Osaka Prefecture (Japan) [July 1996]	Bean sprout	*E. coli* O157:H7 (*stx1* and *stx2*)	12,680 (425; 3.4 %)	12	0.09 %	0/12	[101, 102, 129–131]
Scotland[a] (UK) [Nov–Dec 1996]	Cold cooked meat from single butcher	*E. coli* O157:H7 (*stx2*, phage type 2)	512 (120; 23.4 %)	HUS/TMA 36 (children 6)	7.0 %	17/36 (47.2 %) 17/512 (3.32 %; all deaths >65 years)	[132–134]
Walkerton, Ontario (Canada) [May 2000]	Contaminated municipal drinking water [135]	*E. coli* O157:H7, *Campylobacter jejuni*	Symptomatic Self-reported 2300 (65; 2.8 %)	HUS 30 (Children 22)	1.3 %(total)	6/30 (20 %) 6/2300 (0.26 %)	[136–138]
Germany [2002]	Unknown	SF (sorbitol-fermenting) EHEC O157:NM	Unknown	38	Unknown	4/38 (10.5 %)	[88]
Oklahoma (USA) [August 2008]	Food (diseased food workers in restaurant)	*E. coli* O111:NM (*stx1* and *stx2*)	344 (70; 20.3 %)	25	7.3 %	1/25 (4.0 %) 1/344 (0.29 %)	[139]
Northern Germany [May–June 2011]	Fenugreek	*E. coli* O104:H4 (*stx2*; STEC/EAEC hybrid strain)	3842	855 (children 90)	22.2 %	54/855 (6.3 %) 54/3842 (1.41 %) Pediatric HUS 1/90 (1.11 %)	[63, 105, 140, 141]

[a]There are some discrepancies in the reported numbers between publications
[b]NM (non-motile or H^-)

the effects of the circulating toxin, both locally (HC) and systemically (HUS). Shiga toxinemia appears to occur early during the illness. It is short-lived with an estimated serum half-life of <5 min [146, 147] and has largely ceased when the patient presents with HUS [115]. Of note, coagulopathy, measured as thrombin generation, plasminogen activator inhibitor type 1 (PAI-1) activity, intravascular fibrin deposition and other events can be demonstrated well before – or even in the absence of – the clinical manifestation of HUS [148, 149].

STEC counts and free fecal toxin excretion often diminish or become undetectable during early, acute stages of HUS [115, 150, 151]. The amount of measurable, circulating toxin is extremely low. The difficulty of measuring active toxin in the circulation is likely due to its avid binding to the (microvascular) endothelium, and to plasma proteins or glycolipids. Shiga toxinemia has been demonstrated 24 h after the experimental (oral) inoculation of streptomycin-treated, starved mice with a large dose of the highly virulent Stx2d-producing *E. coli* O91:H21 strain B2F1 [152]. Intravascular binding of circulating toxin by neutralizing antibodies or receptor analogues decreased Stx-induced mortality in these mice when the Stx neutralizing agent was injected during the first 72 h after inoculation [152–155].

STEC: Host Interaction in the Gut

Ingested STEC bind to epithelial cells in terminal ileum and Payer's patches. Bacterial/host cell interaction elicits signals that enhance bacterial colonization and release of bacterium-derived pathogenic factors including lipopolysaccharide (LPS) and Stx. STPB express and excrete various enzymes, additional toxins, such as subtilase, extracellular serine proteases (Esp) and hemolysins [156–168]. Their contribution to hemorrhagic colitis and HUS is subject to ongoing research.

Bacterial Adherence

The first important step in the pathologic process leading to HC and HUS is the adherence of STPB to the intestinal epithelium. Various factors have been identified that facilitate initial attachment. The key set of proteins is described as Type III secretion system (T3SS) [169]. The T3SS is encoded by the locus of enterocyte effacement (LEE); LEE comprises the genes for "intimin," a 94 kDa outer membrane protein involved in intimate enterocyte adherence (*eae*, "enterocyte attachment and effacement"), "translocated intimin receptor" (Tir), a protein injected by attaching bacteria into the gut epithelial cell, and the type III secretion apparatus (Esp B and D). The bacterial proteins work in tandem to form characteristic attaching and effacing (A/E) lesions (actin "pedestals") upon intestinal adhesion (Fig. 26.1a–c). LEE-related genes are shared with related enterobacteriacea, such as enteropathogenic *E. coli* (EPEC) and *Citrobacter freundii* [114, 169–172].

LEE-negative STEC strains [173] use alternative adhesion strategies to host enterocytes, including an iron-regulated gene A (IrgA) homolog adhesin (Iha) [174] and STEC autoagglutinating adhesin (Saa) [175].

Human pathogenic STEC elaborate additional toxins, such as an enterohemolysin (EhxA) [176–179] and subtilase (SubA) [168, 180–182] EhxA, are encoded by a gene located in the pO157 megaplasmid, present in a large proportion of STEC strains [179, 183–185], along with the putative virulence factors EspP that cleaves human coagulation factor V [156], a catalase/peroxidase (KatP) [186], and a metalloprotease that contributes to intimate adherence of EHEC O157:H7 to host cells (StcE) [187].

Shiga Toxin Delivery

The tight adherence of STPB to the epithelium of the gut facilitates toxin translocation into local microvasculature and systemic circulation. Stx binds to Gb3 on Paneth cells and transverses the intestinal barrier without killing the epithelial cell [188, 189]. The microaerobic conditions in the gut reduce bacterial Stx production and release, but enhance Stx translocation across the epithelial monolayer [190]. Inflammatory host response of the gut and resulting diarrhea can be viewed as an attempt of the host to clear out pathogenic bacteria. Interestingly, STEC appear to counteract their removal from the gut by dampening the cytokine response [191].

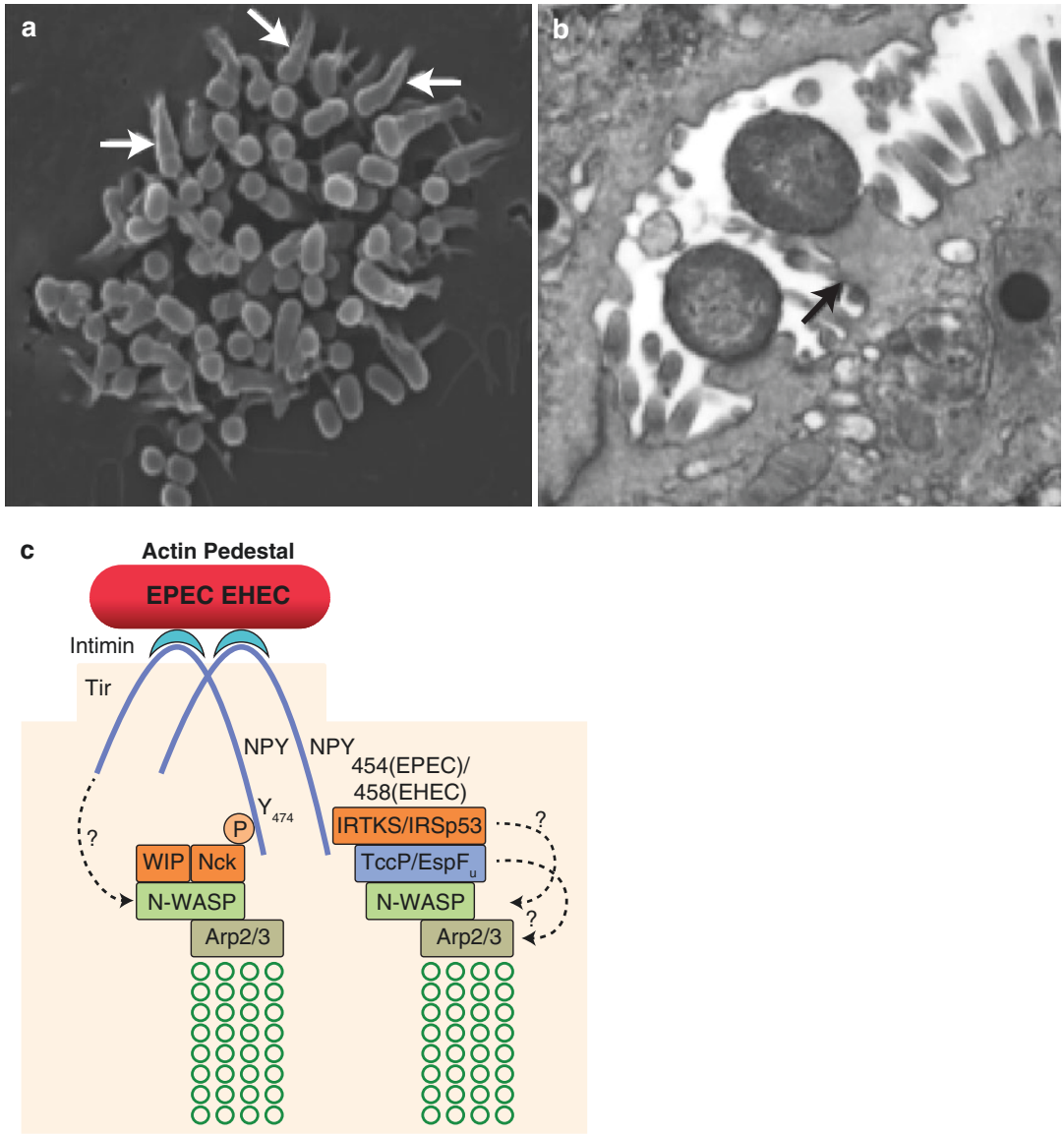

Fig. 26.1 STEC and related attaching and effacing (A/E) pathogens, such as enteropathogenic E. coli (EPEC), induce distinct histopathological lesions using the (bacterial) type III secretion system (T3SS) encoded by the "locus of enterocyte effacement" (LEE). (**a**) Scanning electron micrograph of pedestals induced by adherent bacteria (*arrows*). (**b**) Transmission electron micrograph showing intestinal A/E lesions (*arrow*). (**c**) Diagram depicting the actions of a subset of T3SS effectors of A/E pathogens on host cytoskeletal pathways and structures. *Green circles* represent actin filaments (Used with permission of Jonn Wiley and Sons from Wong et al. [170])

Classification of Pathogenic STEC

Several authors have attempted to classify STEC according to their pathogenic potential in humans [192]. Karmali et al. [193] proposed to group STEC isolates into "seropathotypes" A through E. Seropathotype A is composed of *E. coli* O157:H7 and O157:NM, that are the most common causes of severe STEC disease and outbreaks. Strains assigned to seropathotype B, including O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM, are associated with outbreaks and severe disease but at a lower

incidence compared with seropathotype A strains, while seropathotype E isolates are not associated with human disease [193, 194].

STEC virulence factors are encoded by genes located in mobile genetic elements (prophages, genomic islands, plasmids) that can dramatically alter the virulence of *E. coli* [195]. A revised classification by Kobayashi et al. [194] differentiates STEC strains according to "clusters" 1 through 8 that are defined by virulence gene profiles. In addition to genes located in LEE, the authors identified both *katP* and *stcE* as key attributes of the top pathogenic STEC genotypes. Despite up to 80 % overlap with the seropathotype classification, the proposed "clusters" may more accurately reflect the virulence potential of a given isolate [194].

Stx-Negative STEC

An interesting finding is the loss of *stx2*-containing phages during human disease, originally reported by Dr. Karch's group: Initially *stx*-positive STEC O26:H11/NM and (sorbitol-fermenting) O157:NM patient isolates became *stx* negative during the course of the disease [84, 196]. Similar observations have been reported for STEC O103:H2/NM and O145:H28/NM strains. Thus, *stx* gene content (and Stx production) can fluctuate, with evolutionary, diagnostic, and clinical implications [197].

Shiga Toxin and Its Glycolipid Receptor

Shiga Toxin

Shiga toxins are AB_5 protein toxins consisting of an enzymatically active 32.2 kDa "A" subunit and a (receptor-binding) "B" subunit, which consists of five identical 7.7 kDa proteins. The pentameric B-subunit forms a central pore that anchors the C-terminus of the A subunit (Fig. 26.2a–c). B monomers expose three distinct binding sites that recognize and interact in a lectin-like fashion with the terminal sugars of the glycolipid receptor, globotriaosylceramide (Gb3) [198, 199].

Stx was first discovered in the lysates of cultured *Shigella dysenteriae* named after Kiyoshi Shiga in 1898 [200, 201]. Initially characterized as a neurotoxin, its propensity for endothelial cells was recognized >50 years after the initial publication [202, 203], followed by its description as an enterotoxin in 1972 [204]. Antiserum against Stx from *S. dysenteriae* 1 neutralizes Stx and all Stx1 variants, but not Stx2 or Stx2 variants. In contrast, antibodies raised against Stx2 neutralize most Stx2 variants but not Shiga toxin or Stx1 [24, 35, 205].

Subsequently, numerous *E. coli* strains were characterized that express Stxs with varied amino acid sequences, some of which confer unique biological properties. Because serious outcomes of infection have been attributed to certain Stx

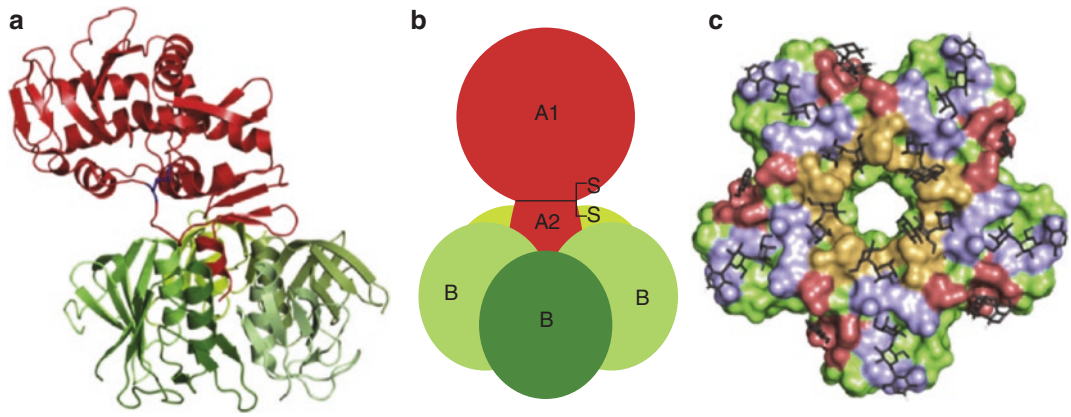

Fig. 26.2 (**a**) The structure of Shiga holotoxin as determined by X-ray crystallography. The A moiety is shown in *red*, the five B subunits in *green*, and the disulfide bridge linking the A1 and A2 fragments in *blue*. (**b**) Schematic representation of the Shiga toxin structure. (**c**) The surface of the B5 pentamer indicating the location of the 15 potential receptor binding sites, based on the structure of Shiga toxin 1 (Used with permission of Elsevier from Bergan et al. [198])

subtypes, an international working group defined the toxin subtypes by comparing the level of relatedness of a large collection of sequence variants comprising three Stx/Stx1 and seven Stx2 subtypes and developed a practical PCR subtyping method [40]. Table 26.1 provides an updated list of members of the Stx "family" associated with human disease.

Shiga Toxin Globotriaosyl Ceramide Receptor

Principles of mammalian cell membrane binding, translocation and downstream effects are identical across all Stxs. The toxins' lectin-like recognition and binding to Gb3 (Galα1-4Galβ1-4Glc ceramide) induces the formation of lipid rafts with clathrin-coated pits that mediate the internalization of membrane-bound toxin into the target cell. Gb3 is identical with CD77 or the P^k blood group antigen [206–209] (Fig. 26.3a, b). There are subtle differences between Stx1 and Stx2 and its variants in their affinity to Gb3 and related glycolipids that influence binding to susceptible tissues and intracellular cell sorting [210–215]. However, these differences do not easily explain why severe disease and HUS are more often associated with STPB producing Stx2 (or Stx1 and Stx2) [216]. Not all Stx subtypes have been isolated from humans with colitis or HUS (Table 26.1).

Gb3 is the only functional receptor for Stx1 and most Stx2 variants in mammals, including humans. Knockout mice for Gb3 synthase, the enzyme that ligates galactose to lactosylceramide (Fig. 26.3a, b), are resistant to the toxic actions of Stx [217, 218]. Expression of Gb3 is

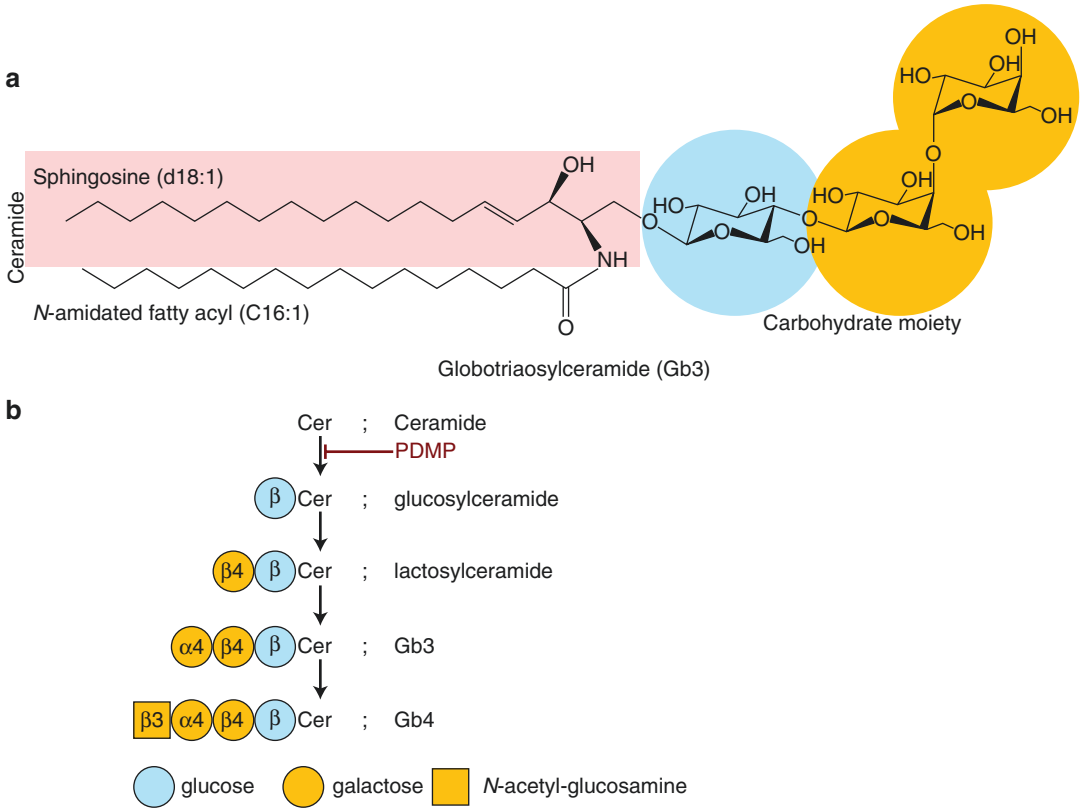

Fig. 26.3 (**a**) Structure of the Shiga toxin receptor Gb3. Glucose and galactose of the carbohydrate moiety are shown in *blue* and *yellow*, respectively. The ceramide moiety consists of a sphingosine backbone (in *pink*) and a variable fatty acid chain. (**b**) The steps in the synthesis of the globo-series of glycosphingolipids from ceramide, showing the sequential addition of carbohydrates represented by the glycan symbol system (Used with permission of Elsevier from Bergan et al. [198])

cell-restricted. It is found on microvascular endothelial cells (including glomerular and peritubular capillary endothelium), glomerular epithelial cells (podocytes), platelets and germinal center B-lymphocytes [219], and peripheral and central neurons [220, 221]. Only the efficient transport to the endoplasmic reticulum and subsequent rRNA depurination result in cell toxicity. Platelets, and possibly red blood cells (RBCs), can bind Stx (and LPS) [208, 209, 222, 223]. Newer ex vivo studies suggest a link between platelet Stx binding, complement activation and microparticle-induced endothelial injury and microvascular thrombosis in patients with eHUS [223–225].

Stx2e, the toxin responsible for porcine edema disease of weanling piglets [226] binds to globotetraosylceramide (GalNAcβ1-3Galα1,4 Galβ1-4Glc Ceramide; Gb4) in addition to Gb3. Gb4 is abundantly expressed in various porcine issues and involved in cerebral vascular injury and neurological disturbance [227]. Although Gb4 is present in human tissues, involvement of Stx2e in human disease is rare and usually mild, likely because *stx2e* phages are mainly found in porcine-restricted pathogens [228–231].

Shiga Toxin: Cellular Biology

The process of Stx binding to the cell membrane and internalization has been described in the 1980s [232–234]. Ongoing research is concerned with details of Gb3 expression and presentation in the lipid bilayer, and intracellular effects [235–240].

Intracellular Toxin Trafficking and Action

Upon its transport across the cell membrane, the toxin subunits disassemble. The "binding" B subunit is marked for ubiquitin-mediated degradation. The A subunit is nicked by the intracellular protease furin [241] and is chaperoned to the endoplasmic reticulum (ER), where the disulfide bond linking the A1 and A2 fragments is reduced. Stx endocytosis and transport to the Golgi apparatus are facilitated by various second messenger (phosphorylation) events that include Stx-induced tyrosine kinases as well as remodeling of cytoskeleton components [198, 242]. The processed A1 fragment then cleaves a specific adenine residue from the 3′ region of the 28S rRNA of the mammalian 60S ribosomal subunit of actively translating ribosomes. Loss of the adenine residue causes a conformational change of the ribosomal RNA resulting in the effective inhibition of protein biosynthesis in toxin-sensitive cells [243] (Fig. 26.4). Very few molecules are needed to paralyze the cell making it one of the most potent known toxins.

The action of Stx on the ribosome not only leads to protein synthesis inhibition, but induces a separable cascade of cell biological effects known as ribotoxic stress response; it is characterized by the activation of the MAP kinase pathway, specifically c-Jun N-terminal kinase (JNK) and p38 [245–252], similar to ricin [253]. The observed biological effects are tissue dependent. For example, bovine intestinal epithelial cells express Gb3 but are insensitive to Shiga cytotox-

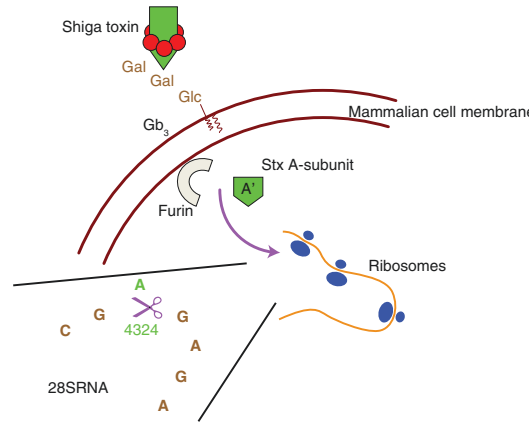

Fig. 26.4 Schematic diagram of the biological action of Stx in susceptible mammalian cells. The holotoxin binds to lipid raft-associated membrane globotriasosyl ceramide (Gb3) and enters the cell via clathrin-mediated endocytosis. A and B subunits become disengaged and the A submit is cleaved and activated by intracellular furin. Upon "retrograde" passage through the Golgi apparatus, the A′ subunit selectively removes a specific adenine residue from the 28S RNA of the large ribosomal subunit (N-glycosidase activity). This results in (1) blockade of active (translating) ribosomes (translational inhibition), and (2) a ribotoxic cellular stress response with activation of c-jun and p38 (MAP) kinases and/or apoptotic cell death (Used with permission of author and Elsevier from Loirat et al. [244]. Copyrighted by Martin Bitzan)

icity, possibly due to their sorting of the toxin to lysosomes instead of the endoplasmic reticulum [254]. Other tissues that are relatively resistant to the cytotoxic effect of Stx may still respond with an "activated" phenotype [255–258]. These effects are in part mediated by an Stx-induced increase in mRNA stability and enhanced protein expression of select (inducible) mRNA transcripts [255, 257–259].

Apoptosis

As outlined above, Stx activates a stress response in sensitive mammalian cells. Intracellular signaling occurs at the plasma cell membrane following Stx binding to Gb3 via the pentameric B-subunit resulting in lipid raft formation, during the course of retrotranslocation of the Stx A subunit, and/or as a result of the purine residue cleavage at the alpha-sarcin/ricin loop of the 28S rRNA [198]. It has become evident that Stx-mediated protein synthesis inhibition is dissociated from cell death signaling and cytotoxicity and that the ribotoxic stress response induced by ribosomal depurination or by the presence of unfolded proteins within the ER (ER stress) induces apoptosis (programmed cell death) [260, 261].

Initiation of apoptosis entails multiple changes to cell morphology, such as cell shrinkage, cytoplasmic vacuolization, chromatin condensation (pyknosis), nuclear fragmentation (karyorrhexis), phosphatidylserine exposure at the plasma membrane, cell blebbing and extrusion of apoptotic bodies [261]. Stx-induced apoptotic signaling is believed to occur mainly via the intrinsic, mitochondrial pathway. It is regulated by B-cell lymphoma protein-2 (Bcl-2) family proteins and results in the downstream activation of cysteine-dependent aspartate-directed proteases (caspases) and the deactivation of anti-apoptotic proteins [198, 248, 261–264]. Pharmacological blockade of MAP kinase and its upstream kinases weakens the Stx-induced cytotoxic (apoptotic) effect *in vitro* [245, 248, 250, 265].

Evidence of apoptosis in renal and other tissues has been demonstrated ex vivo in kidney biopsies from patients with STEC-HUS and in animal models of STEC infection [266–268].

Endothelial Injury in STEC-HUS

Microvascular endothelial cell injury is believed to be the major pathological pathway leading to eHUS. Experimental results from cell culture experiments, animal models of HUS and observations in humans suggest that the endothelium becomes prothrombotic due to injury and apoptotic events. Cultured microvascular endothelial cells, including glomerular and renal tubular endothelial cells, are exquisitely sensitive to nM concentrations of both Stx1 and 2 [269, 270], even in the absence of LPS or TNF-α [271].

The mechanism leading to intravascular hemolysis through the sudden intravascular destruction of RBCs and acute thrombocytopenia is less well understood. Stx (and LPS) interact with platelets, monocytes and neutrophils, and possibly RBCs and plasma proteins (serum amyloid proteins) or glycolipids [205, 223, 225, 272–279]. There is little evidence that Stx modulates neutrophil or monocyte function under *in vivo* conditions [280–284]. The previously postulated "transfer" of toxin from low affinity receptors on circulating leukocytes to high affinity receptors on small vessel endothelial cells [274, 285, 286] may be (too) simplistic [279, 287]. However, investigators have demonstrated more recently that Stx (and LPS) induce the formation of platelet and monocyte microparticles loaded with tissue factor and complement [223, 288]. Biologically active microparticles and (direct) Stx-induced apoptosis of endothelial cells, including the externalization of plasma membrane phosphatidylserine [289], may provide a mechanism how localized (colon) or systemic microvascular thrombosis is initiated in the gut and kidney [290]. Endothelial cell injury or activation may induce or modulate vasoactive mediators, including chemokines and their receptors [255, 258, 259, 290–294] which would then result in a prothrombotic and vasoconstrictive endothelial phenotype known as thrombotic microangiopathy.

The *in vitro* susceptibility of podocytes and of proximal renal tubular cells is similar to that of microvascular endothelial cells [295, 296]. There is a long-standing debate, if tubular injury is a primary feature of eHUS, i.e., directly

Stx-mediated, or secondary to the renovascular (thrombotic) events. Animal experiments suggest that Stx exerts direct effects on renal tubular epithelium [297–299]. Data from the German *E. coli* O104:H4 HUS outbreak support this view [300].

Stx and Cytokines

Stx can lead to enhanced cytokine expression *in vitro* and in experimental mouse models [301, 302]. Conversely, exposure to cytokines, specifically TNF-α, may increase Gb3 in endothelial cells of various vascular beds and enhance Stx sensitivity [303–306]. The release of LPS and (other) exogenous or endogenous inflammatory agents during STEC infection is thought to contribute to or facilitate Stx toxicity and, potentially, the development of HUS [307]. Indirect support for the concept of generalized inflammation is gleaned from the association between HUS risk and margination of peripheral neutrophils (neutrophil count) and acute phase reactants during STEC colitis [129, 308–313] and from *in vitro* and animal experimental data linking the generation of cyto- and chemokines to tissue toxicity.

Data to support the importance of LPS or cytokines in animal models of HUS or Stx-induced renal failure are conflicting [299, 314]. While there is good evidence for direct and indirect cytokine-aided renal parenchymal injury in various forms of glomerulonephritis [315, 316], attempts to detect (increased) circulating LPS, TNF-α or other cytokines in patients with HUS were generally unsuccessful [280, 317–320]. The role of proinflammatory cytokines in the pathogenesis of acute kidney injury (AKI) of human HUS remains inconclusive [300, 306, 321].

Stx Genetics and Stx Phages

Shiga toxins are encoded by bacteriophages (Stx phages) that are closely related to phage lambda (lambdoid) with similar promoters, repressors, terminators, antiterminators, lysis genes, and structural proteins [322, 323]. Lambdoid bacteriophages constitute a heterogeneous group of mobile genetic elements that integrate into specific sites of the bacterial (host) chromosome, with the exception of *stx2e* [228]. The *stx* genes are always located in the same region of these lambdoid phages as part of a late expressed module under the control of anti-terminator Q [324].

Stx phages are lytic and can propagate in receptive *E. coli* and other enterobacteriaceae present in the gut, such as *C. freundii* or *Shigella sonnei* [325–327]. *E. coli* can carry multiple Stx phages leading to the simultaneous production of two or more different Stxs. These conditions are conducive to the creation of novel phages and genome diversification [198, 328]. The genes encoding Stx and enzymes needed for its release are controlled by phage promoters that also regulate the replication cycle of the phage. Phage-mediated bacteriolysis releases the toxin [329, 330]. Stx2 (but not Stx1) can be released from viable *E. coli* using a specific bacterial secretion system [331].

Antibiotics and STEC

Certain antimicrobial and chemotherapeutic drugs induce Stx phage replication and toxin release. This phenomenon has been exploited in the laboratory to increase the yield of Stx for experimental purposes [332, 333], as well as for diagnostic testing of stool isolates [334, 335]. Rare cases of HUS during chemotherapy with mitomycin have been linked to this phenomenon [336–338].

Stx genes are expressed together with bacteriophage SOS response genes [330, 339–341]. Some antibiotics, such as (fluoro)quinolones, sulfamethoxazole/trimethoprim (SMX/TMP) and others lead to prophage induction and Stx production by several orders of magnitude within 2–4 h [342–345]. The SOS response is initiated when damaged bacterial DNA binds and activates the (bacterial) RecA protein. Activated RecA induces the degradation (cleavage) of key repressor molecules, LexA and CI, leading to the temporary arrest of DNA synthesis, cell division and error-prone DNA repair. Cleavage of the CI phage repressor/activator protein results in the coordinately regulated induction and expression of previously silent phage encoded genes (including Stx A and B subunit genes), the production of phage particles, and bacterial cell lysis [330, 340, 345]. Experiments in mice infected with RecA

mutated STEC strains [341] have demonstrated the importance of the bacterial SOS system under *in vivo* conditions.

Antibiotics have been noted to induce an SOS response and phage/Stx expression at levels below minimal inhibitory as well as suprainhibitory concentrations [345]. One study employing subinhibitory norfloxacin concentrations revealed profound effects on the ensemble of the bacterial gene transcripts (transcriptome) of the model *E. coli* O157:H7 strain EDL 933: the vast majority of the upregulated genes was *stx* phage-borne, with up to 158-fold induction of $stxA_2$; conversely, the expression of bacterial genes responsible for bacterial metabolism, cell division and amino acid biosynthesis was down-regulated [346].

Concerns have been raised that the use of antimicrobials that induce an *stx* phage SOS response in patients with (bloody) diarrhea may increase the risk of HUS [347]. Some authors speculated the presence of (putative) triggers of Stx (bacterio)phage in the intestinal lumen may add to the variability of the risk of developing severe hemorrhagic colitis or HUS [344]. The use of antibiotics in this patient population is discussed in detail below.

Laboratory Diagnosis of STEC Infections

STEC infections warrant fast diagnosis; tests must be sensitive, specific and easily accessible. An etiological diagnosis is important for early treatment decisions, particularly by separating STEC-HUS from other HUS forms, and impacts on the long-term clinical follow-up [12, 244, 348]. STEC detection affects close contacts, particularly family and daycare, healthcare institutions, and occasionally schools, restaurants/kitchens and the food industry. STEC infections are notifiable and require isolation measures [349, 350]; results also inform the search for the source of infection and preventive measures to curb further transmission during an epidemic. In the bigger picture, bacterial isolation allows monitoring of epidemiological changes, such as the emergence of new strains and virulence traits.

Identification and management of STEC infection depends on the availability of laboratories testing for STEC and physicians ordering and correctly interpreting results of Stx tests [351, 352]. Current recommendations stipulate that stools are plated simultaneously on an *E. coli* O157:H7 selective agar and tested for the presence of Stx using a fresh stool suspension or overnight broth culture [56, 353]. An Stx immunoassay is not an adequate stand-alone test for detection of STEC in clinical samples [354]. Where available, real-time PCR for the detection of *stx* and other virulence factor encoding genes (if available) in stool or overnight culture should be added. Vero cell or other cell toxicity assays, although highly sensitive and specific, when combined with a neutralization step, are not routinely performed. Stx detection and PCR are important tools for the identification of non-O157:H7 STEC infections.

E. coli O157:H7 are most efficiently isolated by plating fresh stool on Sorbitol MacConkey [SMAC] agar, with or without added cefixime-tellurite [355]. Prior to the use of Shiga toxin assays and PCR, the isolation of non-O157:H7 STEC strains among commensal gut flora by traditional microbiological techniques was laborious, which contributed to the delayed appreciation of non-O157:H7 STEC clones as a cause of enterocolitis and HUS. Another potential barrier to the efficient isolation of non-O157:H7 STEC strains are variable toxin production and instability or loss of toxin producing phages. The latter phenomenon was first noted in subcultures of STEC isolates, but was subsequently shown to occur *in vivo* as well [84, 326]. In fact, the gut appears to be a veritable hot bed for the exchange of phage material and other mobile genetic elements [325, 356].

In addition to STPB identification from stool, testing for (free) fecal Stx is recommended. The classical cytotoxicity assay using Vero or other cell cultures, is time-consuming, labor intensive, and requires cell culture facilities; it has therefore been replaced in most diagnostic labs by ELISA based and other (rapid) diagnostic tests with variable sensitivity and specificity [357–362].

The probability of successfully identifying STPB in children with HUS rises when the first stool samples is tested less than 4 days after diarrhea onset, the patient is 12 months or older, the infection is part of an outbreak, bloody diarrhea is present and onset is during June through September [142]. STEC isolation and free fecal toxin detection rates diminish soon after the onset of HUS [115, 150, 151]. If stool culture or toxin assay(s) are delayed or negative, serological assays can be employed to search for elevated (or rising) IgM class antibodies to one of the more common STEC O-groups (LPS antigens) by ELISA, hemagglutination assay or Western blot [150, 222, 363–366]. Saliva IgA (and IgM) provide a suitable alternative to serum antibodies [366–368]. Testing for serum antibodies to Stx has been used as an epidemiological tool [97, 369], but its diagnostic utility in the clinical setting is limited [142]. Additional STEC-expressed or secreted proteins may elicit an antibody response [370], but are rarely used diagnostically. Serological tests are generally offered in reference laboratories (Table 26.3).

Rapid diagnosis of STEC diarrhea is essential. When sending stools for culture, the clinician should inform the laboratory with clear written and verbal information [372], including the presence of painful or bloody diarrhea, or signs of HUS. "Routine" stool cultures from patients with diarrhea should always include at least a SMAC agar. If the stool culture of an index patient is STEC negative, the pathogen may be identified in other, including asymptomatic, family members. Stools, or broth culture, and serum should be preserved and sent to a reference laboratory in case of negative results, particularly if additional (or suspected) cases of HC or HUS have been identified in the community.

Urinalysis contributes little to the diagnosis of HUS. However, occasionally, a urinary tract infection (UTI) must be ruled out. A patient with moderate to severe hemolysis will present proteinuria and hematuria (hemoglobinuria and erythrocyturia). A documented UTI or the presence of an abnormal urinalysis should not delay the stool-based, etiological diagnosis or the diagnosis of HUS. Macroscopic hematuria is rare in Stx-HUS. Anecdotal reports have described the occurrence of HUS following an STEC UTI without documented diarrhea [373–376].

In conclusion, early stool collection for the culture of *E coli* O157:H7 and, if negative, other STEC serotypes, and testing for the presence of (fecal) Stx should be attempted in all patients with HUS and diarrhea, and in siblings or contacts of index the patient(s). Relying on a clinical diagnosis ("D^+ HUS") and omission of microbiological testing is inadequate. A prerequisite for the correct microbiological diagnosis is the dialogue with the microbiologist. The diagnostic workup should not be limited to free Stx [377]. Where available, stool samples should be screened (by PCR) for Stx genes and/or specific STEC "virulence"-associated genes. In the absence of microbiological evidence of STEC, serological testing for serum or saliva IgM or IgA antibodies against defined O-group (LPS) antigens or other virulence proteins may aid in the diagnosis. Although new and more sensitive diagnostic techniques continue to be developed, a combination of methods will still be necessary for optimal yield [353, 378, 379].

From Colitis to HUS

Clinical Presentation and Evolution

The spectrum of STEC disease ranges from mild diarrhea and hemorrhagic colitis to severe HUS, and death. Fatal outcomes have also been reported in patients with STEC infection without HUS [380, 381].

Most clinical descriptions and risk estimates are based on *E. coli* O157:H7 infections. Severity and incidence of diarrhea or colitis and of HUS by non-O157:H7 serotypes vary due to the heterogeneity of this group of pathogens [65, 81]. The HUS risk is 8–15 % for EHEC O157:H7 colitis [69, 72, 115, 382], but substantially lower for non-O157 STEC infections [111, 113, 383]. The sorbitol-fermenting, Stx2 producing non-motile EHEC O157:NM clone, almost exclusively found in central Europe, appears to be exceptionally dangerous with a HUS risk that exceeds 30 % [88, 120].

The interval between EHEC O157:H7 ingestion and diarrhea is 3–8 days [102, 115, 384]. The diarrhea is typically painful and frequent, with

Table 26.3 Laboratory diagnostic of STEC infections and HUS

STEC disease	Material	Test	Details	Comments
Diarrhea/colitis	Stool	*E. coli* 0157:H7	Sorbitol/tellurite MacConkey agar or similar selective medium)	*E. coli* O157:H7 colonies are distinct due to lack of sorbitol metabolism; tellurite suppresses growth of irrelevant flora
		Free fecal Stx	ELISA, Vero cell tissue culture assay	Fresh stool preferred to prevent decay of toxin protein and activity
		Non-O157:H7 STEC	PCR (for *stx*, structural or phage genes) [40]	Most non-O157:H7 STEC strains ferment sorbitol and cannot be visually or metabolically differentiated on sorbitol containing media
			Colony blot hybridization or blotting	Keep stool sample, colony sweep or broth at -80 °C for reference laboratory
			O-group agglutination	
			Toxin testing of lysates/supernatant	
	Blood	CBC, smear	Baseline hemoglobin and platelets; presence of schistocytes	
		Creatinine	Baseline renal function	
	Urine	Urinalysis	Baseline/early changes	
Acute HUS	Stool		See above	Stool culture may become negative early during HUS
				STPB may loose *stx* phage during course of infection [326, 371]
	Blood	Hematology	CBC, differential, smear, reticulocyte count	
			Consider coagulation screen and d-dimers	
		Biochemistry	Creatinine, electrolytes, albumin, LDH, haptoglobin	Elevated plasma AST and (indirect) bilirubin indicate vigorous hemolysis, not hepatopathy
			Liver enzymes	Detailed complement analysis and/or metabolic or genetic work-up if presentation is "atypical"
			Amylase or lipase	
			Troponin	
			Blood glucose	
			CRP (or other acute phase reactant)	
		Blood bank	Cross and type	
	Urine		Urinalysis	During recovery and follow-up: protein/creatinine
				Acute proteinuria indicates hemoglobinuria with or without glomerular and tubular injury
"Atypical" presentation			Detailed complement analysis and/or metabolic or genetic workup	Relapse (native) or recurrence of HUS (graft kidney)
				Prolonged or waxing and waning course of thrombocytopenia/ hemolysis
				Family history of "asynchronous" HUS

>15 discharges of small amounts of mucous or liquid stools daily; the stools turn bloody by day 2 or 3 in >80 % of children. The amount of (visible) blood varies from a few specks to frank hemorrhage. About 50 % of patients develop nausea and vomiting; fever is present in one third [65, 115, 148, 372, 384]. The evolution of infections by non-O157:H7 STEC serotypes is generally milder compared with *E. coli* O157:H7 [65]. Exceptions are infections by EHEC clones belonging to serogroups O26, O55, O91, O111, among others, that can be clinically indiscernible from infections by classical EHEC O157:H7 [78, 81, 88, 91, 93, 111, 197, 385, 386].

The HUS risk is greatest at the extremes of age, in children <3–5 years and the elderly [115, 132, 380]; it decreases during childhood and adolescence, and is <0.1 % in young and middle aged adults. Conversely, STEC colitis can lead to acute kidney injury (AKI) and death without the picture of HUS, particularly in the elderly [380, 381]. Other variables impacting on the HUS risk are the STEC serotype or clone (as outlined above), the toxin type(s) produced, and preexisting immunity [97, 387]. Experimental animal data indicate that a high Stx "load" exceeding binding to natural protectants and acquired antibodies, is the major precipitant of HUS [152].

Biomarkers and Predictors of Severe STEC Disease

Predictive clinical and biological markers of HUS are the degree of systemic inflammation during the preceding colitis, particularly neutrophilia with a high-percentage shift to band forms, and a sharp rise of acute phase reactants, such as C-reactive protein or calcitonin [102, 129, 309, 310, 312, 313, 388]. Patients who progress to HUS demonstrate significantly more often a peripheral neutrophil count in excess of 20×10^9/L than patients with colitis only [125, 129, 389, 390]. Human and experimental animal data suggest that STEC infection as well as parenteral Stx administration induce a cytokine response that mediates some of the observed pathological effects [293, 301, 391–393]. Some, but not all inbred mouse strains [299] require the combination of Stx and added LPS or TNF-α to produce an HUS-like disease [394–399], while no such dependency on added cytokines was noted in primate models of HUS [392, 393, 400, 401].

Clinical features associated with an increased HUS risk are vomiting, diminished extracellular fluid volume [402, 403] and ingestion of antimotility agents or antibiotics during the first 3–4 days of the diarrheal illness [347, 404]. Female gender, although noted by some authors [68, 405], does not seem to predispose to HUS. The German *E. coli* O104:H4 epidemic represents an exception due to specific features of transmission (vehicle) and consumer habits of many of the victims of this outbreak [140].

HUS mediated by Stx starts abruptly, about 3–10 days (median 6 days) after the onset of diarrhea [115, 129] (Fig. 26.5). Patients present from 1 day to the other with fatigue and pallor; they become listless and may develop petechiae, often after transient clinical improvement of the colitis.

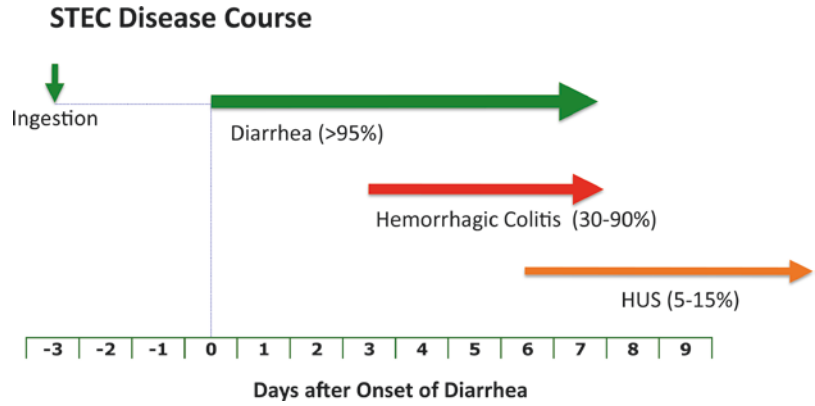

Fig. 26.5 Schematic diagram of the development of diarrhea and HUS due to Shiga toxin-producing E. coli (STEC) (Used with permission of Elsevier from Loirat et al. [244])

Absent bowel movements during the acute phase of HUS should raise the suspicion of intussusception or ileus.

The clinical diagnosis of HUS is generally straightforward, once the HUS-defining triad of (intravascular) hemolysis, thrombocytopenia and acute kidney injury is recognized. Hemolytic anemia of HUS – characterized by RBC fragmentation with schistocytes (burr or helmet cells) in the peripheral blood smear – with or without thrombocytopenia – is known as microangiopathic hemolytic anemia (MAHA). With disease progression, the serum creatinine concentration rises and oligoanuria, hypertension and edema may become apparent, usually within 1–2 days after onset of symptoms. Some patients may recover before the full picture of HUS develops. They may only demonstrate hemolytic anemia without apparent AKI, while platelets may briefly dip within and slightly below the reference range ("partial HUS") [69, 406].

The etiological diagnosis of HUS is important, particularly in patients with atypical presentation, because of the differential prognosis and management of different forms of HUS [12, 348]. Diarrhea has been noted in one third of patients with atypical HUS, occasionally with features of ischemic colitis [407–409]. Conversely, viral or bacterial diarrhea, including STEC infection, can trigger HUS in persons with a genetic defect in the alternative pathway of complement. Such patients should be treated as aHUS [12].

Hematologic Manifestations of STEC HUS

Commonly, the hemoglobin level drops precipitously to <80 g/L with a nadir of <60 g/L. Hemolysis is accompanied by a rapid fall of platelet numbers, usually <50, at times $<30\times10^9$/L. Direct and indirect Coombs (antiglobulin) tests are negative. Rising indirect bilirubin, free plasma hemoglobin and serum lactate dehydrogenase (LDH) (the latter often more than five times the upper normal), and haptoglobin depletion are consistent with a rapid hemolytic process. The peripheral white blood cell (neutrophil) count, already increased during the colitis phase, may continue to rise and, if associated with a leukemoid reaction, can herald a severe course with poor intestinal or renal outcome [68, 308, 410]. The severity of anemia and thrombocytopenia does not correlate with the degree of acute or chronic kidney injury. Some authors noted an inverse relationship between hematocrit (Hb level) and disease severity [410, 411]. A plausible explanation for the latter observation is the hemoconcentration seen in patients with intravascular volume depletion during the first 4 days of STEC colitis [402, 410]. A retrospective study of 137 children with STEC-HUS from Argentina, performed to determine whether dehydration at admission is associated with an increased need for dialysis, indeed showed that "dehydrated" children had a higher rate of vomiting and an increased risk of being dialysed than normovolemic children (70.6 versus 40.7 %, $P=0.0007$) [412].

Thrombocytopenia and active hemolysis usually resolve within 2 weeks. Indeed, a rising platelet count heralds the cessation of active HUS; platelets may transiently rebound to $>500\times10^9$/L. Anemia can persist for weeks after disease recovery without signs of active hemolysis.

AKI in HUS

Renal injury in HUS ranges from microscopic hematuria and proteinuria to severe renal failure and oligoanuria. Up to 50 % of children with STEC-HUS will need acute dialysis [67, 68, 413]. Arterial hypertension is common in the acute phase of HUS and may not be volume-dependent. Blood pressure instability and hypotension with ongoing fever is not a typical feature of STEC-HUS and should raise the suspicion of primary sepsis, complicated by thrombocytopenia and hemolysis, or sepsis from gangrenous (perforating) colitis and peritonitis. Time to recovery of kidney function ranges from a few days to weeks or even months. The risk of long-term renal impairment (CKD, chronic hypertension) increases with the duration of oliguria (dialysis). A commonly cited threshold for the risk of diminished renal recovery is 2–3 weeks [414, 415]. Primary endstage renal disease (ESRD) is rare and should prompt investigations

into a genetic or atypical form of HUS. The results will inform the planning of future kidney transplantation [244, 416].

Extrarenal Manifestations

There is hardly an organ system that has not been affected in STEC infection or HUS. Clinically rare, but important extrarenal and extraintestinal manifestations include myocarditis and (congestive) heart failure, cardiac tamponade, pulmonary hemorrhage, pancreatitis and hepatic involvement, and CNS complications [417–428]. Patients with multiple organ involvement generally also have severe renal injury and often a poor outcome [389, 418, 426]. Postulated mechanisms underlying organ injury in HUS are Stx load and direct tissue toxicity and/or microvascular thrombosis and ischemic injury [418]. Histological and morphological evidence is largely based on autopsy findings (see below).

Rectal prolapse and intussusception may result in transmural bowel necrosis with perforation and peritonitis [429, 430]. In a large study from Argentina, 35 of 987 children with post-diarrheal HUS underwent abdominal surgery requiring bowel resection in 17. Transverse and ascending colon were most frequently affected. Bowel necrosis was noted in 18 and perforation in 12 patients by macroscopic evaluation; histologically, transmural necrosis was present in 21 patients [431].

Serum amylase and lipase activities, considered evidence of exocrine pancreatopathy, are elevated in up to 20 % of patients [429]. Islet injury with transient glucose intolerance or, albeit rare, chronic insulin-dependent diabetes mellitus has been reported [424, 430, 432, 433]. Hepatomegaly and/or rising serum alanine amino transferase (ALT) are noted in up to 40 % of cases; high serum LDH activities may originate not only from RBC lysis, but also from solid tissue ischemia, specifically of liver and skeletal muscle [434]. Acute myocardial insufficiency occurs in less than 1 % of cases [418, 421, 422, 435]. Elevated troponin levels may reflect the degree of myocardial ischemia [436, 437]. Skeletal muscle involvement is exceedingly rare and may manifest as rhabdomyolysis.

The reported incidence of central nervous system (CNS) manifestations varies widely, between 3 % and >41 % [313, 438]. Most case series are retrospective, with variable definitions of CNS injury, frequency and timing of imaging or EEG, or neurological and psychological testing [423, 438–442]. Signs and symptoms can be vague and nonspecific, and are probably underappreciated. Patients may present with irritability, lethargy or decreased level of consciousness; short seizures are relatively common and may reflect fluid and electrolyte imbalances related to AKI and inadequate volume replacement. Abnormal electroencephalograms have been reported in up to 50 % of patients with HUS. Prolonged seizure activity, usually associated with acute respiratory deterioration or palsy, is an ominous sign and may indicate a cerebral stroke or hemorrhage. Acute, transient or persistent (isolated) palsy, dysphasia, diplopia, retinal injury or cortical blindness has been noted [426, 438]. Evident neurological complications or “catastrophic” events are associated with a poor prognosis [426, 439]. Identification of clinical parameters predicting severe neurological events is desirable. A proposed composite score including WBC count, and serum sodium, total protein and CRP concentrations during “early” HUS [441] needs independent validation in a better defined, prospective cohort.

Magnetic resonance imaging (MRI) of the brain is helpful in the differentiation of structural from ischemic or transient injury. However, the predictive value of MRI findings in STEC-HUS remains to be confirmed [427, 443]. In the acute phase, basal ganglia and white matter abnormalities with apparent diffusion coefficient (ADC) restriction are a common and reversible MRI finding. They consist characteristically of bilateral hyperintensities on diffusion-weighted imaging and T2-weighted sequences located in the basal ganglia and thalami, and can extend to the white matter [443]; they can be associated with decreased signal intensity on T1-weighted images of basal ganglia, thalami, and brainstem [427, 428, 443–446] (Fig. 26.6a–f). However, the described changes do not appear to be specific for

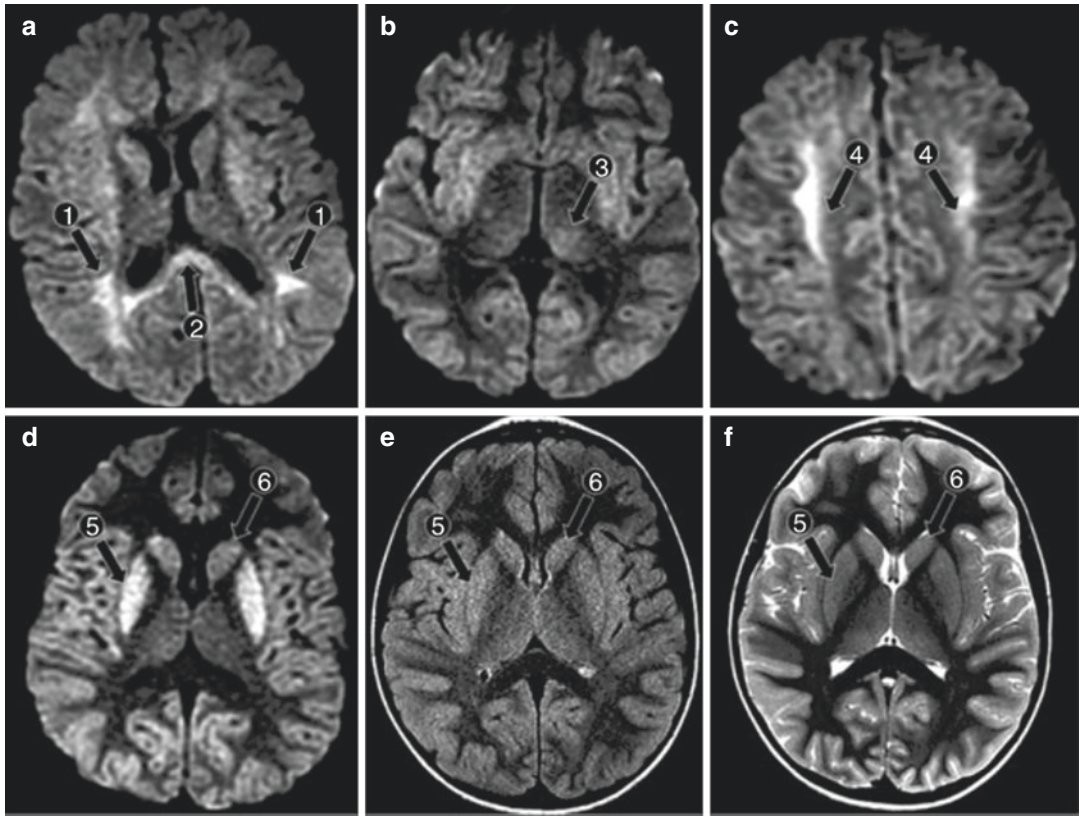

Fig. 26.6 Brain magnetic resonance imaging (MRI) of patients with STEC-HUS acquired within the first 24 h after the onset of neurological symptoms. (**a**–**d**) Diffusion-weighted images (DWI) which demonstrate (*1*) hypersignal involving deep white matter; (*2*) corpus callosum; (*3*) thalamus, (*4*) centrum semiovale; (*5*) putamen; and (*6*) caudate nucleus. (**d**–**f**) Brain MRI of one of the two patients who died. (**d**) The images of this patient demonstrate deep hypersignal on DWI in putamen (*5*) and caudate nucleus (*6*), that can be detected in T2- (**e**) and fluid-attenuated inversion recovery (FLAIR) (**f**) weighted classical imaging. T2- and FLAIR-weighted images of all the surviving patients were normal (not shown) (Used with permission of John Wiley and Sons from Gitiaux et al. [443])

STEC-HUS and often revert over a period of weeks or months [443]. Images can be variable, with or without ADC decrease due to the presence of posterior reversible encephalopathy syndrome or hemodialysis in addition to primary, Stx or STEC-HUS induced lesions [443].

The *E. coli* O104:H4 HUS outbreak in Europe also highlighted the occurrence of psychiatric symptoms [447, 448]. Described manifestations include cognitive impairment [449] and, in a few cases, hallucinations, and affective disorders, such as severe panic attacks. Psychiatric symptoms were associated with higher age ($P<0.0001$), higher degree of inflammation (level of CRP) ($P<0.05$), and positive family history of heart disease ($P<0.05$) [447].

Renal Pathology

Few pathological descriptions are available from renal biopsies of patients with acute STEC HUS [130, 268, 300, 450–452]. Patients with a clinical diagnosis of HUS are not routinely biopsied, except in cases of progressive or chronic renal injury. Most pathological reports have been published prior to 1990, and they do not distinguish between colitis (Stx-) associated and other forms of HUS.

Macroscopically, the kidneys may appear swollen, with numerous petechial hemorrhages on the external surface; on section, the cortex will show areas of hemorrhage and infarction. Focal hemorrhage has also been noted in the collecting system and ureters (Chantal Bernard, unpublished communication).

Prominent *light microscopic* features are the presence of fragmented RBC in *glomerular* capillary loops. Glomerular capillary and renal arteriolar (microvascular) thrombosis may demonstrate prominent fibrin staining, but their extent varies considerably. Endothelial and mesangial cell changes are also evident by *electron microscopy* (Fig. 26.7a–c).

Immunofluorescence is variably positive for fibrin. Immune deposits containing immunoglobulins and/or C1q, C3 or C4 are not a feature of STEC-HUS.

While glomerular histological changes dominate, the *tubulo-interstitial compartment* can also be affected. In fact, all biopsies from a series of patients during the 2011 German HUS outbreak, including those without evidence of TMA, showed severe acute tubular injury [300]. Apoptosis of renal tubular cells has been noted in a few reported cases [266, 452]. Renal cortical "necrosis" and tubular destruction has been shown in cases of clinically severe kidney injury. While the tubulointerstitial changes are reminiscent of a mouse model of Stx-induced renal injury [297, 299], the mechanism underlying these changes has not been determined in human HUS.

Extrarenal Histopathological Aspects

Few histopathological descriptions are available of Stx-mediated changes in extrarenal tissue, mostly post-mortem (autopsy) studies that variably include colon, CNS, pancreas, skeletal and myocardium [450, 451, 453]. These findings demonstrate that STEC-HUS is a systemic disease (microangiopathy) characterized by endothelial cell swelling and injury.

The most consistent changes are demonstrated in the gut: the colon (and rectum) shows diffuse hemorrhagic colitis with mucoal ulcerations and hemorrhagic infiltration of the bowel wall as well as congestion of the serosa and extensive vascular thrombosis. Changes of the small bowel consist of submucosal edema with congested mucosa, with or without intussusception, but may also show the presence of TMA [450]. The pancreas may appear enlarged and swollen, with areas of necrosis and haemorrhage (Chantal Bernard, unpublished observations). Changes in lung and heart may not be specific and may reflect complications prior to death. Descriptions of central nervous system changes in STEC-HUS are scarce. Reported findings consist of brain swelling and bilateral, symmetrical necrotic lesions mainly of the corpus striatum (putamen, globus pallidus) and scattered necrotic lesions in the cortex and other cerebral structures [454].

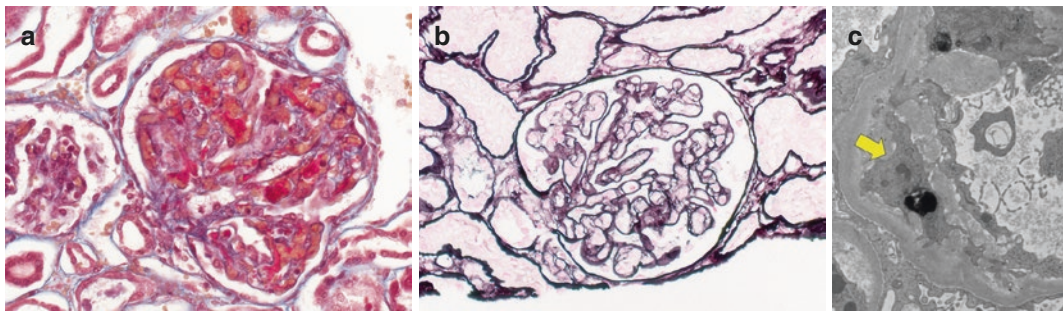

Fig. 26.7 Kidney biopsy, culture-proven STEC-HUS. (**a**) Trichrome stain of glomerulus showing fibrin and RBCs (*orange* and *red*, respectively). (**b**) Silver stain, emphasizing glomerular and tubulular basement membrane structures. (**c**) Electron microscopy; a glomerular capillary is shown. *Arrow* indicates fibrin and proteinaceous material pushing toward the capillary lumen and creating the impression of double contours of the glomerular basement membrane (Courtesy of Dr. Natacha Patey CHU Ste-Justine)

Prevention and Treatment of STEC Disease

There is currently no specific therapy for STEC colitis or STEC-HUS. Nevertheless, best supportive treatment [455] mitigates – if not the development of HUS – at least the severity of the illness and its complications. STEC colitis is a medical emergency [372]. Prompt diagnosis of STEC infection allows initiation of supportive treatment, monitor for signs of HUS, and limit the spread of the organism. In a broader context, the definitive etiological diagnosis, i.e., the isolation and characterization of the infecting organism, is essential for the recognition and control of outbreaks.

Prevention of STEC Infection

Exposure Prophylaxis

Treatment of Stx-HUS starts with the prevention of STEC (STPB) infections. Preventive strategies focus on the implementation of hygienic measures. This applies to cattle farming, the management of drinking water and agricultural produce, and safe practices of food preparation and consumption to containment of the spread of the organism in case of recognized infections [349, 456–459]. The UK Health Protection Agency has established guidelines aimed at the reduction of person-to-person transmission for healthcare providers [460, 461]. The risk of transmission is reduced by adherence to essential hygiene (frequent hand washing and avoiding of touching the face) [458]. Children with proven STEC infection should only return to childcare or school 48 h after the cessation of diarrhea. A thorough review of preventive measures and advice for patients and caregivers can be found in various publications [457, 458, 462, 463].

Vaccines

Active immunization of humans, targeting the O157 LPS antigen [464, 465] and/or Stx and other bacterial antigens [466, 467] remains an elusive goal [468, 469]. Progress has been reported, however, in the vaccination of cattle, e.g., targeting type III secreted proteins, siderophore receptor, porin and intimin [470–472]. Mice and goats, immunized with a promising novel fusion protein, termed Stx2B–Tir–Stx1B–Zot, that carries the immunogenic Stx1 and Stx2 B-subunits, the intimin receptor Tir, and the *Vibrio cholerae* bacteriophage-derived zonula occludens toxin (Zot) which reversibly increases mucosal permeability and acts as mucosal adjuvant, showed substantially reduced colonization and shedding of *E. coli* O157:H7 [473, 474].

Therapeutic Interventions During STEC Colitis

Considerable work has been invested to better understand factors that facilitate the progression from colitis to HUS and to intervene at a stage where the process can be reversed and HUS prevented or at least, ameliorated. Potential strategies are the elimination of STPB from the gut, the binding of free Stx prior to its translocation into the circulation and/or neutralization in the blood stream, thus minimizing the amount of toxin injuring the vascular endothelium. Additional strategies have targeted the coagulation system to prevent microvascular thrombosis and ischemia. Although some of the earlier trials were underpowered, none of these strategies have shown convincing results or promising clinical signals [244, 475, 476]. The conduction of definitive, randomized controlled HUS prevention trials of any intervention in a conventional format is extremely challenging due to the low prevalence of STEC infections and the overall low risk of progression to HUS [153, 403, 477, 478].

Volume Therapy

Volume expansion with isotonic saline administered intravenously during early STEC colitis may ameliorate the severity of HUS. In a retrospective cohort study of 29 unselected children with *E. coli* O157:H7 HUS, Tarr and his group [402] showed that patients who became oligoanuric and needed dialysis had received significantly less intravenous fluid during the first 4 days of diarrhea than children who had preserved urine output and who were not dialyzed. The authors concluded that early parenteral volume expansion before the onset of HUS attenuates

AKI and reduces the need for dialysis. The original findings were reproduced in a prospective multicenter cohort of 50 children with STEC O157:H7 colitis. The treated group received a median volume of 1.7 (0–7.5) vs. 0 (0–4.9) L/m^2 ($P=0.02$) and 189 (0–483) vs. 0 (0–755) mmol sodium/m^2 surface area ($P=0.05$). None of the enrolled patients required ventilatory assistance because of acute pulmonary edema or other volume-related complications [403]. The authors postulated that the oligoanuria of HUS results from renal parenchymal hypoperfusion and ischemia using the analogy of myocardial infarction; and that intravenous volume expansion is an underused intervention that has the potential to decrease the frequency of oligoanuric renal failure in patients at risk of HUS [403]. Support for the "volume hypothesis" comes from an independent study from Argentina [479] and observations linking higher Hb concentrations at presentation with severe HUS, including neurological complications [405, 411].

The administration of isotonic solutions is not expected to affect bacterial toxin production, delivery into the circulation and target tissue binding; however, the intended alleviation of incipient AKI is plausible in view of similar strategies commonly used to prevent or ameliorate acute tubular necrosis and AKI in other scenarios involving potentially nephrotoxic agents [480, 481]. In addition, there are hints that saline infusion may also mitigate the abdominal cramps caused by STEC induced ischemic colitis [482].

Figure 26.8 shows an abbreviated, practical algorithm to estimate the individual child's risk of HUS, initial (minimal) investigations (stool

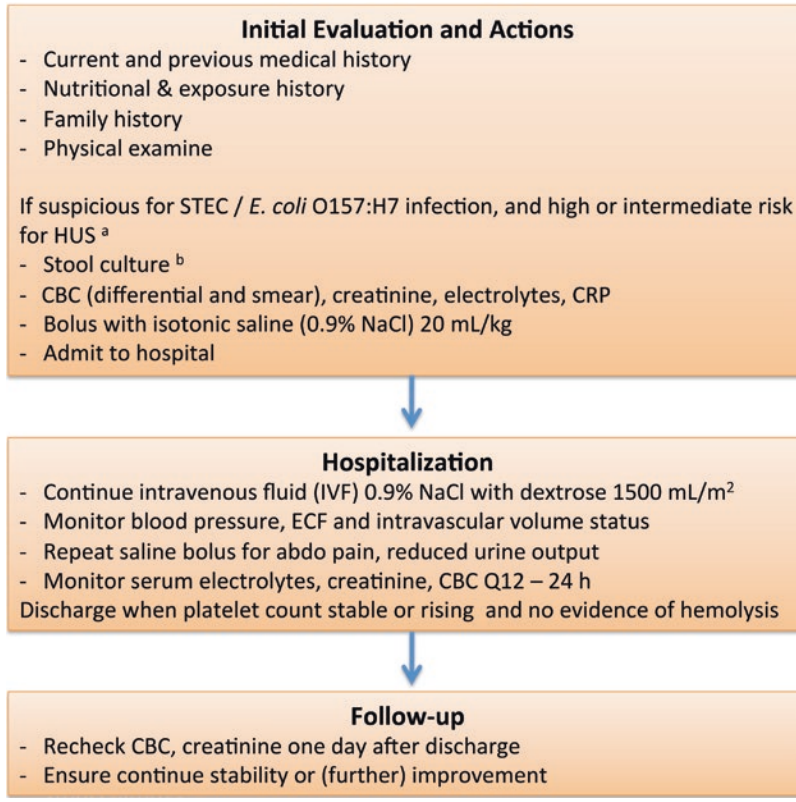

Fig. 26.8 Initial evaluation of STEC infection. (*a*) Risk of Stx HUS: Age group (>6 months) and diarrhea <4 to 7 days that is frequent, turned bloody after 2–3 days, associated with abdominal cramps, or recent HC/HUS in family of community. (*b*) Culture stool for (at least): E. coli O157 (SMAC), Campylobacter, Salmonella, Shigella, Yersinia spp. Stx assay and PCR (for Stx sequences) if available (Used with permission of AGA Institute from Holtz et al. [372])

culture, CBC, renal function, and electrolytes) and the size of the isotonic saline bolus (20 mL/kg body weight) as soon as STEC colitis is diagnosed or suspected [65, 372]. The risk of fluid overload and cardiopulmonary complications due to saline infusion is minimal, provided the patient is hospitalized and supervised diligently by an experienced team [403]. Hospital admission does not only simplify patient monitoring, it may also alleviate parental anxiety and reduce the spreading of the potentially dangerous organisms from the family and community (see above) [349, 350].

Analgesia

Abdominal, typically cramping pain can be severe. Where volume expansion with isotonic saline fails to alleviate the ischemic colitis pain, pharmacological therapy may be warranted. Acetaminophen can be tried, unless there is evidence of hepatopathy. Morphine, found to be effective in children with severe abdominal pain due other etiologies [483], may be administered sparingly, although it tends to worsen post-colitis constipation or ileus. Although there are no separate studies about the effect of morphine in STEC colitis, antimotility drugs in general have been associated with adverse outcome (see below).

Anti-motility Drugs and NSAIDs

Cimolai et al. reported, in a retrospective review of 91 children with HUS from British Columbia, that the prolonged use (>24 h) of a variety of antimotility drugs collectively increased the risk of central nervous system complications, including seizures, encephalopathy or death (multivariate analysis; OR 8.5, 95% $CI_{95\%}$ 1.7–42.8) [405]. A subsequent study of 118 children with STEC colitis (28 with HUS) from the same center revealed that the prolonged use of antidiarrheal agents was associated with development of HUS (multivariate analysis; relative risk 44.1; $CI_{95\%}$ 8.5–229.4) [484]. An independent analysis of 278 children with HUS from the US confirmed the association between antimotility drug use and HUS (OR 2.9; $CI_{95\%}$ 1.2–7.5) [125].

Antimotility drugs do not shorten the duration of diarrhea; their use may prolong bloody diarrhea and increase the risk of systemic complications and should therefore be avoided [125, 485].

Non-steroidal anti-inflammatory analgesic drugs (NSAIDs) have no place in the treatment of patients with STEC colitis or HUS who are often intravascularly volume depleted and at risk of ischemic injury of the gastrointestinal (GI) tract and the kidneys [115, 125, 486–490]. NSAIDs impair renal perfusion and glomerular filtration, and increase the risk of intestinal bleeding.

Antibiotic Therapy of STEC Infection

It is widely accepted that patients with STEC colitis should not be treated with antibiotics. This recommendation is based on clinical observations linking antimicrobial therapy to an increased HUS risk and fatal outcomes [65, 347, 380, 491] and supported by experimental studies demonstrating that certain antibiotics stimulate Stx phage induction and toxin production. Stx induction can occur at levels below or above the minimal inhibitory concentrations of these antibiotics [345, 492, 493].

There is only one published randomized and placebo-controlled trial assessing the efficacy of antibiotics to prevent HUS, by Proulx et al. [494]: 47 children with *E. coli* O157:H7 colitis (mean age 5.3 years, range 3 months to 17.8 years) received TMP/SMX 4/20 mg/kg/dose twice daily (n = 22) or placebo (n = 25) for 5 days. The relative HUS risk in the treatment group was 0.57 (CI 0.09–3.46, $p = 0.67$). The study was underpowered and treatment allocation was not concealed. Importantly, the mean time to treatment initiation was 7 days from the onset of diarrhea, which may have been too late to influence the evolution of the disease (Fig. 26.5) [115, 129].

A multicenter, prospective registry study of 259 children with *E. coli* O157:H7 infection, with laboratory signs of TMA and the occurrence of oligoanuric HUS as primary and secondary endpoints, respectively, showed that children treated with antibiotics during the first week of diarrhea progressed more often to HUS than those who did not receive antibiotics (multivariable analysis; adjusted Odd's ratio 3.62; $CI_{95\%}$ 1.23–10.6). Antibiotic use was further associated with the development of oligoanuric HUS

[347, 404]. When authors compared different antibiotic classes, the association with HUS remained significant for cotrimoxazole and metronidazole, but not for betalactams or azithromycin. Antibiotic therapy also failed to shorten the duration of gastrointestinal symptoms [404]. The latter analysis replicated the results of a retrospective case-control study of an institutional outbreak of STEC O157:H7 HUS, where eight residents developed HUS; five of these eight patients had received TMP/SMX compared to none of the seven residents with diarrhea, whose colitis was not complicated by HUS ($p=0.026$). As the authors point out, antimicrobial treatment may have been given to patients with more severe illness [70].

Two systematic reviews [495, 496] contend that antibiotics confer neither benefit nor a risk of complications. An instructive, prospective cohort study [390] originated from the 1996 outbreak of *E. coli* O157:H7 infection among school children via contaminated bean sprout in Sakai City, Japan, where antibiotics were used extensively during the diarrhea phase [102]. Children infected with the outbreak strain, who were given fosfomycin within the first 2 days of illness (diarrhea), developed HUS significantly less often than those who did not receive antibiotics (adjusted OR 0.15; $CI_{95\%}$ 0.03–0.78). In contrast, fosfomycin started on and after the third day of illness was not associated with HUS prevention [390]. Others suggested a beneficial effect of oral fluoroquinolones compared with IV or PO fosfomycin during the same outbreak [497]. While the latter (retrospective) study has important limitations, it draws attention to the difficulties predicting minimal inhibitory (MIC) and sub-inhibitory concentrations in the (anaerobic) milieu of the gut.

During the enteroaggregative/Stx producing *E. coli* O104:H4 epidemic in Germany, one center chose a deliberately aggressive approach combining intravenous meropenem and ciprofloxacin (and additional oral rifaximin for those admitted to the intensive care unit) [141]. The duration of STEC excretion appears to have been shortened from 22.6 to 14.8 days in patients treated with antibiotics after the diagnosis of HUS [141, 498]. The authors emphasized the presence of less morbidity, including fewer neurological complications (seizures), and a reduced mortality rate [141]. Another cohort study from the same epidemic comprised 65 patients, 22 receiving oral azithromycin and 43 no antibiotics. STEC shedding >28 days was observed in 1 of the 22 treated patients (4.5 %; $CI_{95\%}$, 0–13.3 %) compared with 35 untreated patients (81.4 %; $CI_{95\%}$ 69.8–93.0 %) ($P<0.001$) [498].

It should be emphasized that fluoroquinolones represent the one class of antibiotics consistently found to stimulate toxin production (mainly Stx2) *in vitro* and in experimental animal models, and that little is known about the interaction of co-administered antibiotics. Furthermore, the outbreak strain represents a unique hybrid pathogen (enteroaggregative Stx producing *E. coli*), and most patients described in the above studies were treated after the diagnosis of HUS.

Rifaximin, a semisynthetic derivative of rifamycin with minimal oral bioavailability and proven efficacy in the prevention and treatment of enterotoxic *E. coli* (traveler's diarrhea) [499–501] that interferes with (bacterial) transcription by binding to the β-subunit of bacterial RNA polymerase is an interesting agent for further studies in patients with STEC infection, as is chloramphenicol – although fallen out of favor in Western countries for its rare, but severe hematological adverse effects – due to its suppression of Stx phage induction, *stx2* transcription, and Stx2 production in a number of STPB strains [324].

The potential importance of the interactions of antibiotics and other phage-activating agents *in vivo* has been demonstrated in mouse models of STEC disease. While fluoroquinolones and fosfomycin, given to mice infected with *E. coli* O157:H7, resulted in reduced excretion of the colonizing STEC strain, ciprofloxacin – but not fosfomycin – markedly increased both the presence of free fecal Stx and lethality in the treated animals. Ciprofloxacin treatment also enhanced the transfer of *Stx2* prophage to other *E. coli* the gut [502].

In a mouse model of STEC encephalopathy, oral administration of a single azithromycin dose 2 h after the oral instillation of a 100 % lethal

infective dose of the Stx2c-producing STEC strain E325211/HSC protected all animals. Using the same model, fosfomycin, ofloxacin and ciprofloxacin failed to protect STEC infected mice, while kanamycin and norfloxacin improved survival, albeit less effectively than azithromycin [503]. Similar results were shown in the gnotobiotic piglet model of oral Stx2 (but not Stx1) producing STEC O157:H7 infection [504].

Novel, non-antibiotic agents are on the horizon and may offer an alternative strategy. One such experimental compound is phenethyl isothiocyanate (PEITC), a dietary anticancer compound derived from common vegetables [505, 506]. It has been shown *in vitro* to suppress STEC growth, phage induction and Stx production by effecting a stringent bacterial response mediated by massive production of a global regulator, guanosine tetraphosphate (ppGpp) [507].

Consensus on the use of antimicrobial therapy in children with hemorrhagic colitis is lacking and the hypothetical benefit of antibiotics in the prevention of STEC HUS remains controversial. Controlled, prospective studies with select antimicrobial agents are necessary to resolve this important caveat [508, 509]. Rifaximin and few other agents, such as azithromycin or fosfomycin may be suitable to limit the spread of the organism and, potentially, to reduce the rate of complications during STEC epidemics. Based on the experience during the Sakai outbreak in Japan, the antibiotic should be given immediately, i.e., within 48 h after the onset of diarrhea to be effective [390]. Only agents that do not induce a bacterial SOS response or augment Stx production at any concentration should be used [141, 324, 510]. All other antimicrobial agents should be discontinued after STEC identification if already started [115, 509, 511]. Until additional data become available, caution is advised in prescribing antibiotics empirically to children with bloody diarrhea, except in *Shigella dysentereriae* endemic regions.

STEC-HUS: Therapy and Management

The presence of diarrhea ("D+ HUS¨) is not synonymous with STEC (Stx, enteropathogenic) HUS; a rigorous search for the etiology of any HUS is important for therapeutic decision-making and the prognosis. Any patient with a second episode of HUS or with an "asynchronous" family history of HUS and (thorough) exclusion of STEC infection (Table 26.3) should be screened for genetic mutations of complement regulatory and related proteins as well as for ADAMTS13 activity, and presumptively diagnosed and treated as "atypical HUS" [12]. As the recent discovery of a (homozygous) mutation in the gene encoding the protein triacylglycerol kinase epsilon (*DGKE*) among patients with infantile aHUS showed, additional causes of (a)HUS will be identified with time.

Current Therapeutic Approach and Best Supportive Care

Symptomatic treatment of manifest HUS follows general principles established for patients with AKI, however with some specific recommendations related to the often rapid hemolytic process and thrombocytopenia. All patients need careful monitoring of vital signs, fluid intake and excretion, and signs of cardiac, respiratory and neurological deterioration. Treatment focuses on the stabilization of vital functions, intravascular volume status, acid-base, serum electrolytes (potassium, sodium, calcium and phosphate) and uric acid. Patients may develop pleural or pericardial effusion, or cardiac insufficiency, in addition to potentially serious complications of the colitis, requiring careful monitoring and intervention. As with other critically ill children, nutrition – already compromised during the diarrhea phase – is part of the treatment.

In the wake of the German 2011 HUS outbreak, "best supportive care" for patients with HUS was defined as "volume replacement, parenteral nutrition and dialysis" [455, 508]. This care is best provided in a center with experienced (pediatric) nephrologists where extracorporeal purification techniques and a Critical Care environment can be provided around the clock. Referral to such a center early in the disease course is strongly recommended.

Management of Hematological Manifestations

The large majority of patients with HUS receive packed red blood cells [413]. The threshold for PRBC infusions is clinically defined: symptoms (tachycardia and tachypnea) and/or the velocity of intravascular RBC destruction as gauged by frequent Hb determinations, rising LDH and free plasma Hb levels. A practical cut-off is a Hb of 60 g/L (hematocrit <18 %). Twelve to 15 mL of PRBC per kg body weight can be transfused over 2–4 h; a loop diuretic can be given in case of fluid overload or hyperkalemia (such as furosemide 0.5 mg/kg IV). If necessary, PRBC transfusions can be timed to coincide with hemodialysis sessions, particularly if hyperkalemia or volume overload are a concern, or when a blood prime is needed for (small) children undergoing HD. PRBCs should be deplete of leucocytes and platelets, as practiced in most pediatric hospitals. Transfusion of PRBC and platelets has to be weighed against the HLA alloimmunization risk, particularly in children with severe AKI who may not recover kidney function.

Erythropoiesis-stimulating agents (ESA) may provide a benefit beyond the reduction of PRBC transfusions [512]. However, a more recent study from South America failed to demonstrate a difference in the number and frequency of blood transfusions following the use of ESA [513].

Platelet transfusions during the acute HUS phase should be limited to (rare) active bleeding and to (some) surgical procedures. A reptrospective, single-center cohort analysis of children with acute HUS requiring dialysis access, revealed no difference with respect to bleeding complications or catheter survival in patients who did (30 %) or did not receive platelets (70 %) prior to the procedure [514]; the authors concluded that peritoneal and central venous catheter placement can be accomplished safely in most children with HUS, without a need for platelet transfusion, despite the associated thrombocytopenia. Previously expressed concerns that transfused platelets accelerate microvascular thrombus formation and promote tissue ischemia as reported in adulthood TTP [515, 516] have not born out in practice [479]. Platelets may be concentrated (volume-reduced) to avoid fluid overload; however, most oliguric patients will need dialysis, making this a lesser concern.

AKI Management in HUS

AKI treatment pertains to the management of fluids and electrolytes, hypertension and nutrition. About 50 % of children with eHUS will need some form of renal replacement therapy.

Fluid and Electrolyte Management

Assessment of intravascular volume status helps guide initial treatment toward fluid replacement or restriction, and administration of diuretics or dialysis. Patients are monitored for fluid intake and changes in urine output, along with frequent measurements of BP and heart rate. Weight changes correlate poorly with effective circulatory volume. Intravascular volume may be decreased secondary to intestinal losses and reduced oral intake during the early phase of the disease and may result in hypoperfusion of the kidneys. Third spacing, especially in the gut, and generalized edema – due to endothelial injury and capillary leak – may mask intravascular depletion and, if not corrected, aggravate ischemic injury. Patients warrant diligent intravascular volume expansion to improve organ perfusion, particularly of the gut, kidneys and brain. Systemic hypotension is rare and should raise suspicion of an alternative diagnosis or secondary sepsis, e.g., from gangrenous colitis or line infection.

Fluid restriction may be necessary in patients with fluid overload secondary to oliguric renal failure. If intravascular volume is replete, a trial with furosemide at a dose of 1–2 mg/kg may be attempted to induce diuresis and delay dialysis, particularly in patients with pulmonary or cardiac compromise, or to bridge the time to catheter placement.

Aggressive challenge with high-dose loop diuretics has been advocated in the past to prevent progression to oligoanuric failure and avoid dialysis [517]. However, clinical trials and meta-analyses of studies in patients with AKI due to a variety etiologies, primarily in intensive care

settings, have failed to document a beneficial effect of furosemide on short or long-term renal outcome [518–520].

It seems best to follow a pragmatic path and use a loop diuretic to bridge the time to safe hemo- or peritoneal dialysis catheter placement while avoiding harm (toxicity, intravascular volume depletion). General indications to initiate RRT are electrolyte disturbances including hyperkalemia, hyperphosphatemia and metabolic acidosis; fluid overload unresponsive to medical treatment; and symptomatic azotemia. Some investigators believe that hyperuricemia contributes to renal function deterioration and discuss the benefit of removing uric acid [521, 522].

Antihypertensive Therapy

Arterial hypertension occurs commonly in acute (STEC) HUS. It may be caused by renal microvascular thrombosis, Stx-mediated, direct vascular endothelial cell injury or activation, or intravascular fluid overload. Cerebral complications may contribute to hypertension. Conversely, systemic hypertension can lead to CNS complications, such as posterior reversible leukoencephalopathy syndrome (PRES) [523–526] or facial palsy [527]. PRES and co-existing cerebral vasoconstriction syndrome may also represent manifestations of similar underlying pathophysiologic mechanisms [528].

Although activation of the renin-angiotensin system (RAS) has been invoked [529–531], several clinical and genetic studies have failed to demonstrate a relationship between plasma renin activity and the course of HUS. Specifically, activation of the RAS in HUS was noted to occur irrespective of hypertension [532]. While the interpretation of renin measurements is complicated by physiological, age-related differences [533], hypertension in HUS does not appear to be directly linked to RAS activation. Furthermore, earlier studies also noted that the BP did not correlate well with the estimated degree of hydration in children with HUS [529].

Where antihypertensive therapy is needed during the acute phase of HUS, it is reasonable to use dihydropyridine calcium channel blockers (nifedipine, amlodipine or PO/IV nicardipine). While acute treatment with ACE inhibitors has to be balanced against concerns over the impairment of renal perfusion and hyperkalemia, RAS blockers are a rationale choice for patients with HUS-induced chronic hypertension with or without residual CKD or proteinuria [534–536].

Renal Replacement Therapy

Up to 50 % of children with STEC-HUS will need dialysis treatment [102, 413, 537, 538], but higher rates, up to 70 %, have been reported in registries and select HUS outbreaks [81, 91, 105]. The North American Synsorb Pk® (Synsorb Biotech, Calgary, Canada) trial (Synsorb Biotech, Calgary, Canada) protocol mandated that dialysis be delayed until 72 h post diagnosis of HUS, if clinically acceptable to the responsible physician. Under these restrictive conditions, 39 % of the 49 placebo-treated patients were dialyzed for a mean of 3.6 days [413].

There is no evidence that early dialysis changes the evolution of acute eHUS or long-term outcome. However, delaying dialysis unduly increases the risk of complications of kidney injury. Indications for dialysis initiation in HUS are similar to those for other causes of AKI and may evolve rapidly: severe electrolyte imbalance (hyperkalemia, hyperphosphatemia), acidosis or fluid overload refractory to medical/diuretic therapy, symptomatic uremia and, possibly, hyperuricemia. The presence of profound thrombocytopenia, anemia and (rare) gangrenous colitis will influence the choice of dialysis modality and anticoagulation.

Dialysis Modality

The choice of the dialysis technique depends on patient-related, clinical and practical aspects, such as availability of HD and adequately trained personnel, patient size for (central) access creation, local preference and experience, particularly when dialyzing young children and infants. Factors favoring PD are young age (infants), lack of pediatric-trained HD facilities, and avoidance of anticoagulation. Diarrhea or colitis is not considered a contraindication for PD [153, 413].

Continuous Renal Replacement Therapy (CRRT)

CRRT is rarely needed, but may offer an alternative approach to conventional dialysis in children who present contraindications to PD or where PD has failed. HUS patients with severe fluid overload, cardiovascular instability, with or without sepsis, and multiorgan failure may benefit from CRRT. "Slow" hemodialysis offers an alternative where CRRT is not possible. Both are typically performed in a critical care setting. During the acute stage, when patients are thrombocytopenic, HD and CRRT can be tried with minimal heparin, provided there is sufficient blood flow. Regional, citrate-based anticoagulation offers an alternative to heparin, specifically in patients with cerebral stroke or hemorrhage, or after surgery.

Apheresis Modalities

Plasma Exchange (PE) Therapy

Some pediatric centers consider severe, life-threatening STEC-HUS an indication for PE as "rescue" therapy, especially in patients with CNS complications [153, 539]; this approach may have been influenced by the success of PE in patients with TTP (see below). However, the benefit of PE in eHUS remains unproven [476, 540–542], unless aHUS is suspected [348]. The European Paediatric Study Group for HUS excluded other forms, specifically STEC-HUS, from this form of invasive therapy [348]. In young children, PE necessitates the insertion of a large-bore central venous access (hemodialysis line), which confers additional morbidity to children not already undergoing HD [409].

Although PE has no proven benefit in children with STEC-HUS, it is commonly used in the treatment of adult patients. Reports of its efficacy in (adult) patients with STEC-HUS are limited to uncontrolled observations, including data published after the Scotland outbreak [133, 543–546], and none of the more recent (adult) recommendations to initiate PE [543, 544, 546] is based on controlled trials [508]. Nevertheless, large-scale PE administration occurred during the *E. coli* O104:H4 epidemic.

One reason for this variation in practice between pediatric and adult nephrologists is the traditional view of HUS as part of the TTP spectrum ("HUS/TTP") [547–549]. The previously dismal prognosis of (classical) TTP [550], now known to be caused by anti-ADAMTS13 (auto) antibodies [551–553], has improved dramatically with the introduction of intensive PE therapy (and immunosuppression) [554, 555].

A careful, comprehensive analysis of the treatment strategies during the *E. coli* O104:H4 HUS outbreak concluded that PE failed to improve the outcome of adult HUS patients [141], Furthermore (and in keeping with the pediatric experience [409]), prolonged PE was found to be potentially harmful [104, 476]. Consequently, the latest, 2013 guidelines on the use of apheresis by the American Society for Apheresis (ASFA) categorized STEC-HUS as a disorder where apheresis treatment is ineffective or harmful (category IV) with 1C evidence [556].

Hypothetically, PE would remove inflammatory mediators, bacterial toxins and plasma microparticles, and – if performed against frozen plasma – replenish coagulation and complement factors [557]. However, the concentration of Stx in the circulation amenable to apheresis is minute due to rapid toxin binding and uptake by endothelial cells. Some authors noted that PE removes platelet- and leukocyte-derived plasma microparticles [288], while others associated the procedure with increased microparticle generation [558, 559]. Although the concept of microparticle removal may be appealing for specific diseases, the pathological importance of (prothrombotic) particles and the benefit of their removal for the outcome of HUS is currently far from clear.

Extracorporeal Immunoabsorption

Immunoabsorption (IA) is a specialized apheresis technique aimed at the removal of IgG from plasma by specific binding to a column loaded with (purified or recombinant) staphylococcal protein A; it has been advocated by some centers for the treatment of HUS associated with severe CNS disease. In a recent, uncontrolled series, the authors speculated that pathogenic (autoimmune

?) antibodies may be responsible for the neurological symptoms in STEC-HUS [560]. While this hypothesis remains to be tested, the usually rapid clinical resolution of acute neurological signs unrelated to detectable infarction or hemorrhage with and without IA (or PE) argues against this speculation.

Antithrombotic and Antiplatelet Agents

Treatment with anticoagulation/antithrombotic agents (heparin, urokinase, or antiplatelet drugs such as aspirin or dipyridamole) has failed to ameliorate the course of HUS or to decrease the mortality rate, neurological events or long-term sequelae [476, 561]. These (negative) findings correspond to experimental Stx-HUS in primates [562, 563]. On the contrary, heparin and antithrombotic agents have been associated with an increased risk of bleeding in at least two trials (RR, 25.9; $CI_{95\%}$ 3.7–183) [476]. However, these earlier studies were underpowered, and interventions started late during the course of the disease. Without a beneficial "signal," none of the latter agents can be currently recommended in the treatment of STEC-HUS [153, 469, 476].

Non-medical Supportive Therapies

Nutritional Support

Appetite and nutritional intake are already limited when infants or children present with HUS due to the preceding colitis, with or without vomiting. Patients usually continue to experience abdominal pain, likely due to ischemia, often with constipation, partial ileus or persistent colitis. Nausea and inability to eat can be accentuated by uremia, surgical intervention and opiates given for pain relief, or by PD. Nutritional support with nasogastric feeding or total parenteral nutrition should be initiated early in the course of the disease. Insulin therapy may be required in case of endocrine pancreatic involvement.

Psychosocial Support

Patient and familial anxiety and (valid) concerns about the disease process, prolonged hospitalization, repeated invasive procedures and the prognosis of the HUS – paired with information in the lay press and the internet, specifically during outbreaks – warrant the support by a social worker or psychologist.

Experimental Therapies and Novel Mechanisms of STEC-HUS

Shiga Toxin Neutralizing Agents

The concept of binding Stx in the blood stream or, preferably, prior to its translocation from the gut into the circulation, is not new. Receptor analogues, e.g., for TNF-α (etanercept), or toxin neutralizing antibodies (e.g., *Clostridium botulinum* or *C. tetani* toxin antibodies) are well established treatment modalities. Animal studies support the feasibility of this approach in STEC disease, at least in an experimental setting [153].

Stx Receptor Analogues

Predicated on its effective toxin binding *in vitro* and a reduction of the fecal toxin load in experimentally infected mice [564, 565] synthetic Gb3 linked to an inert, non-resorbable carrier (Synsorb-Pk®) has been studied in a randomized controlled North American (US/Canada) trial [413]. The investigators hypothesized that the agent, administered orally soon after the diagnosis of HUS, would diminish continued toxin absorption and result in disease amelioration. Primary endpoints were decreased rates of death, serious extrarenal events and dialysis frequency. The trial was stopped when the interim analysis revealed no difference between treatment and placebo groups. Several consideration may explain the negative trial outcome: (a) the binder may not reach the site of toxin production and delivery by STEC that adhere tightly to the mucosa via "attaching and effacing" lesions [566], (b) the binding affinity and capacity may have been insufficient and/or the chosen dose too small, and (c) the timing after HUS onset may have been too late. New, multi-branched (clustered trisaccharide), high-capacity oral and systemic Gb3 analogs (e.g., "Starfish," "Daisy," "Super Twigs") [567–570], peptides that prevent intracellular Stx targeting when bound to the circulating toxin [571–573], and genetically modified, Gb3-expressing *E. coli* and (other) probiotics

are being developed [574–576], but no new clinical trial initiatives have been announced or published [154, 155].

Shiga Toxin Antibodies

In murine and newborn piglet models of STEC-HUS, infusion of toxin-neutralizing monoclonal antibodies up to 3 days after orogastric infection protects against hematological and renal disease, but efficacy decreases rapidly with delayed antibody administration [152, 577]. No important adverse effects were noted when the anti-Stx2 antibody TMA-15 (Urtoxazumab) was infused in children with documented STEC colitis [578]. Another phase II trial (NCT01252199, "Shigatec") with a monoclonal antibody combination against Stx1 and 2 (Shigamabs®, Thallion Pharmaceuticals Inc.) [579–581] showed likewise good tolerance [478]. Further development and clinical testing of each antibody product was stalled due to corporate decisions. Newest developments include the generation of highly effective single domain antibodies that recognize and neutralize Stx1 and 2 [582].

The theoretical window for meaningful intervention is narrow. It is still debated if relevant toxin absorption from the gut continues, once HUS has become manifest, and whether toxin neutralization at that stage has any ameliorating effect on the disease course and outcome.

Considering the low incidence of STEC-HUS and the multicentric logistic infrastructure needed to conduct a traditional randomized controlled trial as demanded by the FDA (in 2006) and other regulatory agencies (http://www.fda.gov/ohrms/dockets/ac/07/minutes/2007-4286m1.pdf), despite its orphan drug status (http://www.medscape.com/viewarticle/521133), makes it unlikely that anti-toxin antibodies will become available anytime soon for patients with STEC infection at risk of HUS.

Anticomplement Therapy in STEC-HUS

Complement and STEC-HUS

The potential role of the complement system in the pathogenesis of STEC-HUS gained traction over the past few years. Several events stimulated scientific inquiry and clinical interest: progress in the understanding of atypical HUS caused by genetic or acquired dysregulation of the alternative pathway; availability of a potent, well tolerated anti-complement (anti-C5) antibody (eculizumab); and the eruption of the *E. coli* O104:H4 epidemic in Northern Germany which coincided with a report in the New England Journal describing three children with STEC-HUS and severe CNS involvement who recovered after the infusion of eculizumab [583].

Clinical Observations

Reports of decreased plasma C3 concentrations in children with presumed STEC-HUS appeared since the 1970s [584–586]. The first reports predated the recognition of the central role of STEC infection in children with (typical) HUS. Several authors described glomerular deposition of C3 in (kidney) biopsies obtained during the acute phase of HUS [587]. Nonetheless, the majority of eHUS patients demonstrate C3 serum levels within the reference range. The employment of more sensitive assays confirmed the activation of complement in eHUS. For example, Monnens et al. detected elevated levels of breakdown products of the two components of the alternative pathway C3-convertase, C3 (C3b, C3c, C3d) and CFB (Ba) in the serum of children with "post-diarrhea" HUS [585]. More recently, increased plasma levels of the CFB activation product Bb and the soluble form of the terminal complement complex sC5b-9 were demonstrated in 17 children with acute STEC-HUS that normalized by day 28 of the disease [588]. Comparable results were obtained in a Swedish cohort of 10 children, all of whom displayed elevated C3a and sC5b-9 (terminal complement complex) concentrations in plasma as well as C3 and C9 bearing, mainly platelet-derived microparticles (measured as mean fluorescent intensities) during acute STEC-HUS that normalized with disease recovery [223]. A detailed case study of one patient with acute eHUS by the same authors revealed the presence of C3, Stx2 and LPSO157 on a larger number of platelet-neutrophil and platelet-monocyte complexes (by flow cytometry) compared with healthy controls [223]. The cited findings suggest the recruitment of complement components, together with Stx and bacterial LPS, on the surface of

platelets [589] and, to a lesser degree, neutrophils and monocytes, and alternative pathway activation in acute STEC-HUS [223]. Stahl et al. speculated that while complement activation is not the primary event occurring during EHEC infection, it may contribute to blood cell activation and renal injury, through the release of microparticles, free radicals and cytokines. Deposition of the terminal complement complex (TCC or sC5b-9), demonstrated in post-mortem tissue from a child with STEC-HUS (Fig. 26.9a–f) further suggests that complement is activated in this disease.

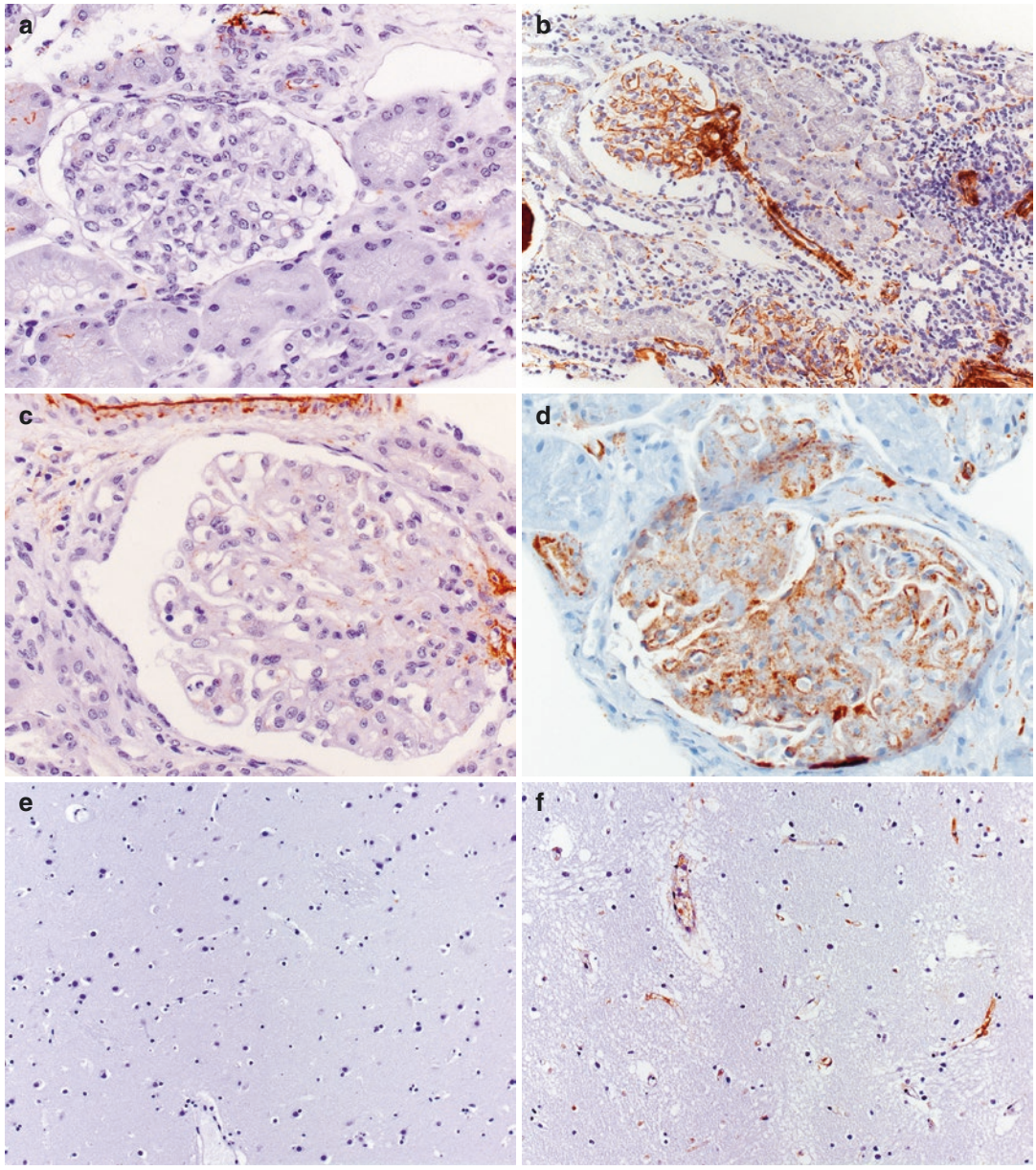

Fig. 26.9 C5b-9 (membrane attack complex, MAC) deposition in kidney and CNS sections of a child who died of STEC O157:H7 HUS. (**a**) Control (kidney). (**b**) Strong staining of renal arteriole and glomerular capillaries with anti-MAC antibody (1:200). (**c**) Staining of afferent and efferent arterioles, minor staining within the glomerulus. (**d**) Ubiquitous glomerular capillary staining with anti-MAC antibody. (**e**) control (brain). (**f**) Staining of cerebral capillaries with anti-MAC antibody (Courtesy of Dr. Natacha Patey CHU Ste-Justine)

Interestingly, no correlation was found between the levels of activated complement products in plasma or on platelet derived microparticles and the presence or absence of renal or extrarenal complications [223, 588].

Experimental Studies

The hypothesis that complement is activated in STEC-HUS has been addressed by several laboratories. Morigi et al. reported that Stx1 induced cultured microvascular endothelial cells to express the membrane adhesion molecule P-selectin which bound and activated C3 (via the alternative pathway). The resultant C3 cleavage generated C3a, a potent chemokine, which conversely upregulated P-selectin expression and thrombus formation under flow conditions, along with a reduction of endothelial cell thrombomodulin (TM) expression [399]. This study confirmed results previously published by others using glomerular endothelial cells [590].

In this animal model, Stx may directly contribute to complement activation: C3 was found to be deposited when Stx1-treated microvascular endothelial cells were exposed to normal human serum [399]. Perfusion of Stx-treated endothelial cells with whole blood led to increased surface thrombus formation compared to monolayers not exposed to toxin; thrombus formation was prevented by the complement inhibitor sCR1. Endothelial complement deposition and loss of thromboresistance was dependent on Stx-induced P-selectin expression and high-affinity C3 binding and alternative pathway activation [399, 591], and was blocked by a P-selectin antibody and the P-selectin soluble ligand PSGL-1. The findings were reproduced in the authors' mouse model of HUS induced by the combined injection of Stx2 and LPS [399]. The absence of CFB (injection of genetically modified mice deficient of CFB with Stx2 and LPS) mitigated the degree of thrombocytopenia and protected against glomerular microangiopathy and renal function impairment, further supporting the involvement of alternative pathway of complement activation in this HUS model [399].

Another mechanism underlying activation in patients with STEC-HUS is the interaction of Stx and LPS with platelets and leucocytes as outlined above [223]. *In vitro* experiments by the same group showed that soon after incubation of human whole blood with purified Stx1 or Stx2 or O157 LPS, platelet-leucocyte complexes and platelet-leucocyte-derived microparticles bearing complement C3 and C9 on their surfaces became detectable, along with the generation of C3a and soluble C5b-9 complex. This effect was enhanced in the presence of LPS compared with Stx alone [223].

Based on experimental studies showing that Stx2 binds CFH, specifically the short consensus repeats (SCRs) 6-8 and SCRs18-20, domains required for CFH surface recognition [592], Würzner and Orth promoted the idea that interference with this functionally and quantitatively most important inhibitor of unregulated AP activation causes – or contributes to – complement activation in STEC-HUS [593]. The same laboratory subsequently demonstrated that CFHR-1 and CFHL-1, complement proteins with partial homology to CFH, also bind Stx2, and compete with Stx2 binding to CFH [593, 594],

The above experimental and human data are at variance with the results reported by Kurosawa and Stearns-Kurosawa's group in Boston, who employed a non-human primate (baboon) model of Stx-TMA that closely resembles human HUS. Importantly, in this model HUS is induced by the infusion of purified Stx without the addition of LPS or TNF-α [400]. To their surprise, the authors failed to discover significant increases in soluble terminal complement complex levels (TCC or SC5b-9) after lethal challenges with Stx1 or Stx2 [595]. Complement activation and disseminated intravascular coagulation (DIC) were, however, evident in baboons with sublethal *E. coli* bacteremia. Complement inhibition with compstatin, a peptide that prevents C3 cleavage and activation, reduced consumptive coagulopathy, inflammation and microvascular thrombosis in the bacteremia model, and improved cardiac and renal function [596, 597]. It should be remembered, however, that bacteremia is not a feature of (typical) childhood STEC-HUS.

The complement system is a fundamental bacterial defense mechanism [595]. Human HUS is characterized by STEC-induced colitis with

intestinal injury, hemorrhage and leucocytosis that may contribute to complement activation [595], likely involving C3a and C5a [597, 598]. Further research is warranted to identify complement activation pathways and effectors in human HUS and the therapeutic benefit of various anti-complement agents [595, 599]. Currently available evidence does not support the general use of complement blockers in the treatment of STEC-HUS [141, 600–602].

Thrombomodulin (THBD) and Complement Activation

THBD (CD141) is a multidomain, constitutively expressed endothelial integral membrane type-1 glycoprotein. It acts as receptor for the serine protease thrombin and mediates anticoagulant and antifibrinolytic properties of the endothelium. In addition, it dampens the inflammatory response through its anti-inflammatory properties [603, 604]. TM can also negatively regulate the complement system by accelerating factor I-mediated inactivation of C3b [604, 605].

Inactivating mutations of TM, although exceedingly rare, have been identified in patients with aHUS [606], both in isolation or combined with mutations of cognate complement proteins. Apart from its role in aHUS, evidence is emerging for an involvement of TM in STEC-HUS. Experimental data indicate that TM expression is decreased in Stx2 exposed human glomerular microvascular endothelial cells that had been sensitized with pro-inflammatory mediators [590]. In the Bergamo mouse model of HUS, induced by the combined injection of Stx2 and LPS, reduced glomerular TM expression was observed in association with fibrin and platelets deposition [399]. Zoja et al. further demonstrated that mutation of the lectin-like TM domain worsened murine Stx-HUS [607]. Mice lacking the lectin-like domain exhibited excess glomerular C3 deposition indicating impaired complement regulation and local generation of complement-derived, pro-inflammatory peptides, such as the chemokines C3a and C5a. Binding of C3a to endothelial cells leads to impaired endothelial thromboresistance and may contribute to the TMA in the authors' model [399].

Soluble, recombinant TM (rTM) has been administered successfully to septic patients with DIC [608]. Honda et al. reported the use of rTM-α in a small, uncontrolled series of pediatric patients with severe STEC-HUS [609]. While the authors observed clinical resolution and favorable disease outcome in all three cases, efficacy and benefit of this novel therapeutic agents remain to be established.

Membrane-Bound Regulators of Complement Alternative Pathway

Experiments using cultured glomerular endothelial and immortalized human proximal tubular cells, revealed that Stx "down regulated" the expression of CD59 (protectin), but not of the decay-accelerating factor (DAF, CD55) or membrane cofactor protein (MCP/CD46) [610]. Studies in survivors of *E. coli* O104:H4 HUS, examined after recovery, showed increased CD59 expression on granulocytes and monocytes. The same group noted, however, abundant CD59 on the patients' RBCs that were studied during the acute HUS [611].

Therapeutic Complement Blockade in STEC-HUS

Eculizumab has been tried as "rescue" therapy in a few patients with severe neurological manifestations of HUS due to *E. coli* O157:H7 infection. The authors of the first publication detailing this approach reported rapid neurological improvement, which was unexpected considering the natural history of this complication, along with the cessation of hemolysis and normalization of the platelet count [583]. All three patients recovered without neurological or renal sequelae. The generalizability of these encouraging finding remains unclear. Several pediatric and many adult patients with HUS received eculizumab during the *E. coli* O104:H4 epidemic, mainly on compassionate grounds. Careful analysis of a large cohort from this HUS outbreak comparing the clinical findings and outcomes of patients receiving eculizumab versus "best supportive care", with or without plasma exchange failed to demonstrate that complement blockade with the anti-C5 antibody (or plasma exchange therapy) changed the course of the disease [141].

In the absence of a controlled trial with predefined endpoints, it is currently premature to recommend anti-complement therapy for the treatment of (typical) STEC-HUS. Exceptions are patients with a severe, fluctuating or prolonged course of "diarrhea-associated" HUS with or without proven STEC infection: some of these patients may indeed experience an exaggerated or uncontrolled activation of the alternative pathway of complement induced by Stx or other STEC-derived proteins [223], or by an underlying (or unrecognized) genetic defect of the complement or coagulation cascade, including thrombomodulin [612, 613],

In conclusion, experimental and ex-vivo studies suggest that Stx may interact directly with CFH and CFH-related proteins, or induce alternative pathway of complement activation, secondary to endothelial cell injury, and interfere with endothelial thromboresistance. The putative roles of TM and complement-derived chemokines require additional studies both to better understand the pathogenesis of this form of HUS and to design effective therapeutic interventions. However, there is no doubt that Stx itself can injure the microvascular endothelium and trigger the activation of various cellular and plasmatic cascades, including the platelet/coagulation and complement systems. With a few documented exceptions [612–614], STEC-HUS patients do not have underlying complement regulator deficiencies or autoantibodies, nor does the generally acute and self-limited course justify treatment with anticomplement agents [141].

Outlook and Future Research

Currently, there is no specific therapy to prevent or treat STEC-HUS. The testing of new therapeutics for the prevention or mitigation of (severe) HUS poses important challenges due to the relatively rare occurrence and the acuity of the disease. Such studies require an innovative trial design and international collaborative efforts. Candidate therapeutic agents that may be tested in the future include, among others, fluid therapy, (intravenous) toxin binders (Gb3 receptor analogues, Stx-neutralizing antibodies), recombinant thrombomodulin and complement inhibitors (anti-C5 antibody, recombinant complement receptors), and select, non-phage inducing antibiotics during the early diarrhea phase.

Complications and Long-Term Outcome

Acute Prognosis

STEC-HUS represents an important cause of AKI in young children. Its course is generally self-limited. Hematological improvement (decreasing LDH activity, rising numbers of platelets in the circulation) may herald the beginning of renal recovery, but normalization of kidney function typically lags a few days to weeks behind the resolution of thrombocytopenia and hemolysis.

Overall, the outcome of STEC-HUS has improved substantially since its first description [1, 615, 616]. Reported mortality rates vary between <1 % and 5 % (and up to 12 %; median 1–4 %) [617], mostly secondary to CNS or cardiac involvement, or catastrophic colitis. About 70 % of patients recover completely from the acute episode; the remainder suffers varying degrees of sequelae [389, 410, 422, 423, 426, 430, 435, 617, 618].

While early diagnosis and supportive intervention, such as fluid management during the colitis phase and better dialysis techniques may have improved outcome, shifts in endemic STEC clones and the occurrence of epidemics may confound comparisons between periods. Sustained oligoanuria, a WBC $>20\times10^9$/L and hematocrit >23 % were found significantly more often in patients who died during acute *E. coli* O157:H7 HUS (or later, due to severe complications) than in survivors [132, 410]. A large registry study from central Europe identified seven deaths among 490 enrolled, EHEC-positive patients (1.4 %; $CI_{95\%}$ 0.01–0.04) during the acute phase (2–30 days after diagnosis; median age 3.6 years [IQR, 2.7–4.7 years]). All seven patients had been dialyzed; five presented neurological manifestations, including three who died of cerebral edema [91]. The authors of another series of

children with STEC-HUS from France reported a lethality of 17 % among patients with CNS complications [426].

Complications and mortality rates appear to be greater in adult patients than in children with HUS. Lethality of STEC-HUS in the elderly population may be as high as 50 % [132, 140, 619, 620].

Long-Term Outcome

Despite its importance, the long-term renal prognosis of patients with HUS is controversial [621]. There is consensus that children who have recovered from the acute phase of HUS are at risk of long-term complications including CKD, arterial hypertension, neurological impairment or diabetes mellitus [410, 622, 623]. Long-term renal complications have been reported in 5–25 % of patients ranging from persistent microalbuminuria or proteinuria and hypertension to CKD and ESRD secondary to nephron loss. Fifteen to 30 % of patients demonstrate (usually mild) proteinuria and 5–15 % arterial hypertension; chronic (CKD) or endstage renal disease has been noted in approximately 10 % of surviving patients [91, 389], and ESRD in 3 % [617].

A carefully conducted, prospective long-term follow-up study of the drinking water *E. coli* O157:H7 epidemic from 2000 by the Nephrology group at the University of Western Ontario (Walkerton Health Study), found that 5 years after the event, 20 % of pediatric HUS survivors had microalbuminuria; the HUS cohort had an average 10-mL/min/1.73 m^2 decrease in GFR compared with age-matched controls from the same community [621]. The observed prognosis was better than reported in other studies; none of the children with HUS had overt proteinuria or GFR less than 80 mL/min/1.73 m^2 (1.33 mL/s/1.73 m^2) or blood pressures higher than expected for community norms.

Possible reasons contributing to the discrepancies found in the literature are sampling bias and loss of follow-up, differences in the infecting STPB strains (single outbreak scenarios, i.e., homogenous exposure, versus variable strain exposure in spontaneous cases). Comparison between reported outcomes is also hampered by the lack of clear definitions [617]. The cited European registry study [91] defined "poor" outcome as the presence of arterial hypertension (systolic or diastolic blood pressure >95th percentile according to age, sex, and height), neurological abnormalities (seizures, coma, stroke, or delayed motor development), impaired renal function (estimated glomerular filtration rate [eGFR] <80 mL/min/1.73 m^2), or presence of proteinuria (positive dipstick analysis or protein-to-creatinine ratio >0.15 g/g). Based on these criteria, 30 % of 274 prospectively followed patients were found to have sequelae 5 years after the initial diagnosis: 9 % of the study population had persistent hypertension, 4 % neurological symptoms, 18 % proteinuria without or with (7 %) decreased eGFR. Of importance, hypertension and proteinuria was noted in 18 % of patients who had been free of renal sequelae at the end of the first year following HUS ($CI_{95\%}$ 0.12–0.26) [91].

Predictors of Long-Term Renal Outcome

The risk of long-term renal complications is commonly thought to be related to the duration of anuria (or dialysis) during the acute phase of HUS. Frequently cited cut-offs are 10 or 14 days of oligoanuria [415, 617]. However, substantial overlap exists, and individual predictions are prone to errors [415]. For example, Rosales et al. noted that the duration of dialysis correlated with the risk of presenting with sequelae at the 1-year, 2-year, and 3-year follow-up ($P = 0.0004$; Odds ratio [OR] for each 1-day increase in dialysis period, 1.04–1.08). Patients presenting with long-term complications were dialyzed for a median period of 15 days (IQR, 7–22 days) compared with 9 days for those with favorable (uncomplicated) outcome (IQR, 6–14 days; $P = 0.01$) [91].

Registry studies from Utah [414, 415] and from the UK [624] noted that up to one-third of children with severe HUS (defined as anuria >8 days and oliguria >15 days) developed long-term sequelae. Oakes et al. analyzed a cohort of 159 children with HUS from 1970 to 2003 and at least 1 year of follow-up (mean 8.75 years) [415].

The authors of the latter study defined oliguria as urine output <240 mL/m^2/day, and anuria as urine output <15 mL/day. In this cohort, 90 children (57 %) had at least 1 day of oliguria and 69 (43 %) at least 1 day of anuria. The occurrence of chronic sequelae (proteinuria, low GFR [estimated GFR <90 mL/min/1.73 m^2], hypertension [BP >95th percentile for height and age]) increased stepwise with the duration of anuria, markedly with >5 days of anuria or >10 days of oliguria with anuria performing better as a predictor of sequelae than oliguria. Hypertension was present in 55.6 % of patients with >10 days of anuria versus 8.9 % in those without anuria (OR 12.8; $CI_{95\%}$ 2.9–57.5). Anuria >10 days was associated with combined GFR loss and proteinuria in 44 % versus 2.2 % of patients without anuria (OR 35.2; $CI_{95\%}$ 5.1–240.5). On the other hand, 36 % of children with no recorded oliguria or anuria were left with sequelae, and 10 % of those with no recorded oliguria or anuria were found to have proteinuria [415].

Histological studies of kidney biopsies from patients with HUS suggest a correlation between the extent of glomerular microangiopathy and long-term renal prognosis: the prognosis was noted to be poor when >50 % of glomeruli were affected and in the presence of arterial microangiopathy and/or cortical necrosis [451]. However, a biopsy is rarely performed during the acute stage.

Outcome of Extrarenal Manifestations

Up to 30 % of children with CNS manifestations during the acute phase of HUS develop long-term neurological sequelae [423]. Subtle neurological problems attributable to HUS are probably underdiagnosed, such as learning and behavioral difficulties, reduced fine motor coordination, or attention deficit and hyperactivity disorder [625].

Long-term gastrointestinal complications after STEC-HUS are colonic strictures and bilirubin gallstones, insulin-dependent diabetes mellitus, exocrine pancreatopathy [429, 430, 432, 433].

Myocardial insufficiency, attributed to previous eHUS, has been described [421, 422, 626].

Post-HUS Monitoring and Long-Term Interventions

Delayed renal function deterioration >1 year after the HUS has been reported in patients who appeared to have completely recovered [91, 415, 627]. Furthermore, a normal GFR does not exclude nephron loss with compensatory hyperfiltration in the surviving nephrons. Rosales et al. found late-onset hypertension and (chronic) proteinuria in 18 % of their cohort [91]. Although not formally studied, angiotensin converting enzyme (ACE) inhibition is a rationale therapeutic strategy in patients with hypertension and/or proteinuria, with the aim to reduce glomerular pressure [621]. Yearly evaluation of kidney function, blood pressure and urinalysis (with quantitative urine protein or albumin measurements) has been recommended for at least 5 years, and indefinitely for patients with renal sequelae and/or hypertension [91, 424, 617].

STEC-HUS and Kidney Transplantation

Consistent with the observation that most patients with STEC-HUS recover kidney function after the acute episode, the number of patients with kidney transplantation following this form of HUS is limited. Of 274 STEC-HUS patients followed longitudinally through the German-Austrian HUS registry [91], seven patients (1.4 %) required chronic dialysis and received a kidney allograft (one patient had developed ESRD within 2 months, five within the first year, and one 2 years after disease onset).

Renal transplantation in patients with STEC-HUS is safe without specific precautions, as demonstrated in several case series and registry reports [91, 628]. In a series from Argentina, no difference was observed between pediatric transplant recipients with and without a history of HUS with respect to graft survival and function, number of rejections and patient survival over up to 20 years; none had evidence of HUS recurrence [628, 629].

However, there is a caveat to the general principle. A few cases have now been described where STEC-induced HUS led to ESRD and subsequent recurrence of HUS in the graft [416, 613, 630]. Detailed genetic screening of two of these cases demonstrated the presence of disease-associated

mutations of complement regulator genes [613]. As emphasized elsewhere, a second episode of HUS, even with microbiologically proven STEC infection, and HUS recurrence in the graft, indicate another (primary) etiology. These patients cannot be classified as "typical HUS" or (typical) "STEC-HUS", and genetic complement regulator abnormalities should be suspected and searched for [416].

Shigella dysenteriae HUS

Clinical Presentation and Epidemiology

HUS is a rare, but recognized complication of *Shigella dysenteriae* type 1 (SD1) infection. The first traceable descriptions of HUS following shigellosis appeared in the 1970s [631–634]. While some of its features resemble those of STEC-HUS, the reported disease course is generally more severe. The account of 81 cases of HUS during the 1994–1996 SD1 epidemic in South Africa shows acute oliguric renal failure in 90.1 % and dialysis in 42 (51.6 %). Disseminated intravascular coagulation (DIC) was recorded in 21 % [635]. Additional complications are listed in Table 26.4.

The age range at presentation is wide (median 3 years). HUS is diagnosed more than a week (range 4–17 days) after the onset of bloody diarrhea (colitis), and occasionally after diarrhea has improved. Reported incidences of HUS related to all dysentery cases range from 6 % to 45 % (median 13 %) [636]. However, much lower figures emerge from prospective cohort studies [637] (Table 26.5).

Pathogenesis of *Shigella dysenteriae* HUS

Unlike STEC, *S. dysenteriae* is an invasive organism that penetrates the bowel wall, enters the blood stream and spreads hematogenously. Patients with SD1 infection should be treated with appropriate antibiotics. Shiga toxin (Stx) is likely involved in

Table 26.4 Organ involvement in *Shigella dysenteriae* (SD1) HUS

Organ involvement	Details	Percentage (of SD1 HUS cases)[a]
Generalized	Septicemia	18.5
	Disseminated intravascular coagulation (DIC)	21.0
	Hyponatremia	69.1
	Hypoalbuminemia	82.7
Gastrointestinal	Toxic megacolon	4.9
	Gastrointestinal perforation	9.9
	Protein-loosing enteropathy	32.1
	Rectal prolapse	6.2
	Hepatitis	13.6
Renal	Oliguric AKI	90.1
	Dialysis	51.6
Central nervous system	Encephalopathy	37.0
	Convulsions	14.8
	Hemiplegia	2.3
Heart	Myocarditis	6.2
	Congestive cardiac failure	3.7
	Cardiomyopathy	3.7
	Infective endocarditis	1.2
Hematological	Leukemoid reaction	91.3

[a]Extracted from Bhimma et al. [635]. Percentages are from 81 of 107 cases of HUS, admitted between July 1994 and February 1996, following an outbreak of *S. dysenteriae* type 1 dysentery in Kwazulu/Natal

Table 26.5 Pediatric *Shigella dysenteriae* type 1 (SD1) HUS

Country	# of reported cases	Age in years (mean or median)	Interval between onset of diarrhea and diagnosis of HUS (days)	Case fatality rate (%)	References
South Africa	151	4.6	7	17	[635, 638–640]
Zimbabwe	110	1.5	11	41	[641, 642]
India	74	2.3	8	59	[643, 644]
Nepal	55	2.1	17	23	[645, 646]
Saudi Arabia	33	3.0	8	26	[647, 648]
Bangla Desh	30	3.3	6	37	[649]
Kenya	21	1.6	4	52	[650]

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the pathogenesis of *S. dysenteriae* colitis and HUS. The release of lipopolysaccharide (LPS) during the invasive infection is thought to potentiate the ribotoxic effect of Stx and disease severity [651, 652]. Children with SD1-HUS can demonstrate impressive neutrophilia and left shift [635, 641]. As with STEC-HUS, a high peripheral neutrophil count has been associated with the development and severity of HUS [649, 653]. Some patients manifest the full DIC picture [3, 654]. Although STEC can induce subtle coagulation activation [148], full-blown DIC is not seen in STEC-HUS in the absence of gangrenous or perforating colitis and secondary sepsis or peritonitis.

Morbidity and mortality of SD1-HUS appear to be substantially higher than of HUS due to STEC infection [636]. The estimated case fatality rate is 36 % and by far exceeds that of shigellosis without HUS [635–637, 641, 655]. According to some authors, HUS has emerged as the principal cause of death in epidemics of SD1 dysentery [636]. However, *S. dysenteriae* is endemic in areas with limited resources and access to health care, and affected children may be malnourished and suffer from additional morbidities [656]. Severe fluid volume loss, hypernatremia and lack of dialysis may contribute to the previously reported poor outcome, described particularly in Sub-Saharan epidemics.

Antibiotic Therapy and HUS Risk in *S. dysenteriae* colitis

Antimicrobial therapy is the standard of care for patients with shigellosis. SD1 Stx is encoded in a defective lambdoid prophage unable to become lysogenic, which leads to constitutive Stx production [25, 637, 657, 658]. Thus, exposure of *S. dysenteriae* type 1 to antibiotics is not expected to increase Stx expression via phage induction. However, bacterial killing by antibiotics and subsequent lysis could augment stool toxin concentrations. In an attempt to estimate the risk of HUS attributable to antibiotic use, investigators at the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh analyzed a well-defined group of children with SD1 infection who were treated early in the course of illness. Free fecal Stx levels decreased after the administration of antibiotics in 85 % of enrolled children, and none of the studied patients developed HUS [637].

The same authors reviewed the results of seven shigellosis drug trials, most of them performed in Bangladesh, during 1988–2000. Antimicrobials were administered within 96 h of the onset of dysentery. A total of 378 patients had proven SD1 infection (66 % children) [637]. The list of antibiotics used in these studies comprises nalidixic acid, ciprofloxacin, cefixime, ampicillin and other betalactams, and azithromycin. A single child, treated with ciprofloxacin, developed HUS, corresponding to a calculated risk of 0.004 (95 % $CI_{95\%}$ 0.001–0.022) in children and of 0.0026 (95 % $CI_{95\%}$ <0.001–0.015) in all participants. For persons with *S. dysenteriae* type 1 colitis, early administration of effective antimicrobial agents, including fluoroquinolones, is associated with decreasing Stx concentrations in stool and a low risk of HUS [637, 659].

Non-SD1 Shigellosis and HUS

Severe intestinal disease and extra-intestinal manifestations occur with infections by any of the four Shigella species, but most commonly with *S. dysenteriae* type 1. In a study of hospitalized pediatric patients <15 years old in Dhaka, Bangla Desh, *S. flexneri*, *S. boydii* and *S. sonnei* were significantly less likely ($P<0.05$) to cause grossly bloody stools (33 versus 78 %), frequent stools in the 24 h before admission (median 11 vs. 25), rectal prolapse (15 versus 52 %), or extra-intestinal manifestations, including leukemoid reactions (2 versus 22 %), severe hypernatremia (26 versus 58 %) and neurologic manifestations (16 versus 24 %) [656]. The same authors calculated an incidence of HUS of 1 % by non-SD1 *Shigellae* versus 8 % by *S. dysenteriae* type 1. Death rates due to infections by any of the four *Shigella spp*. were similar (10 %). Factors significantly associated with death were younger age, lower stool frequency before admission, poor nutrition, hyponatremia, documented seizure and unconsciousness [656].

Streptococcus pneumoniae HUS

Epidemiology

Invasive pneumococcal disease (IPD) may lead to HUS, variably referred to as pneumococcal (pnHUS, pHUS) or *Streptococcus pneumoniae* HUS (SpHUS). It occurs in <0.6 % of IPD episodes [660] and affects mostly infants and young children with a median age at presentation of 13 months (range 5–39 months) [661]. Authors from New Zealand reported a 10-year cumulative incidence rate of 1.2 per 100,000 children under 15 years ($CI_{95\,\%}$ 0.5–2.0) [662]. The majority of patients (three fourths) present during the cold season (October to March in the Northern hemisphere) [661, 663].

pnHUS accounts for approximately 5 % of all pediatric HUS, and 40 % of all non-STEC HUS cases in children [663, 664]. Relative incidence estimates from the Canada, the UK and New Zealand range from 3 % to 11 % of all HUS cases [661, 662, 665]. Indigenous populations, such as Maori and Pacific Islanders may have a higher disease burden than other groups [662].

While increased vaccine coverage may have led to a decline in the incidence of pnHUS, *S. pneumoniae* serotype 19A has emerged as the predominant isolate during the last decade [661, 666–668]. Serotype 19A is missing in the first generation pneumococcal 7-valent conjugate vaccine (PCV7 [Prevnar®, Pfizer Inc., New York, NY]; serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) [669], but has been incorporated into the current 13-valent vaccine (PCV13; pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) [670] and the 23-valent pneumococcal polysaccharide vaccine (PPV23; Pneumovax®, Kenilworth, New Jersey, USA).

Pathogenesis

More than 85 years after the discovery of the T agglutination phenomenon [671], and more than 40 years after the report linking microangiopathic (intravascular) hemolytic anemia and HUS with *S. pneumoniae* infection and *in vivo* neuraminidase production [4], the pathogenesis of pnHUS is only partially understood, and the approach to treatment remains controversial [244, 672, 673].

pnHUS typically develops in a patient with pneumonia, often with pleural empyema (70 %), or meningitis (up to 30 %) [661, 668, 673, 674]. HUS has been linked to abundant in situ production of bacterial neuraminidase [4, 675], in particular neuraminidase A (NanA) [676]. Neuraminidase cleaves terminal sialyl (*N*-acetyl neuraminic acid, Neu5Ac) residues from membrane glycoproteins and glycolipids of red and white blood cells, endothelial cells, and other tissues [676–678]. The exposed O-glycan core 1 (Gal β1-3 GalNAc α-O-) is known as (asialo)glycophorin A [660, 679] or Thomsen-Friedenreich disaccharide (T or TF antigen) [671] (Fig. 26.10). The TF antigen is recognized by the lectin *Arachis hypogaea* which has been used to detect the *in vivo* effect of neuraminidase on RBCs and tissues in patients with pnHUS [4, 5, 676] and to

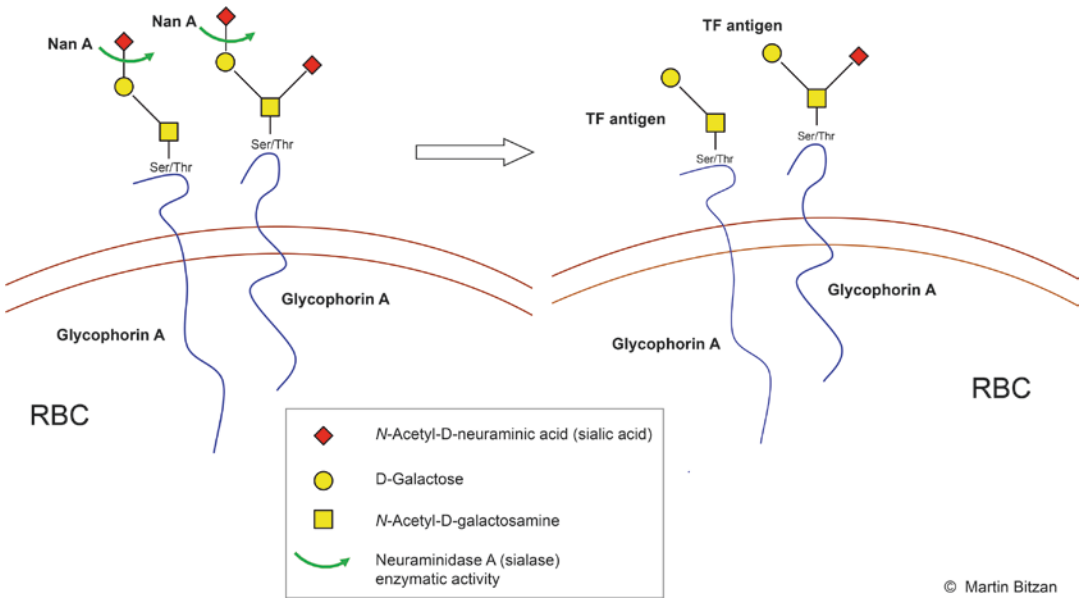

Fig. 26.10 Neuraminidase action on RBC membrane. Nan A (pneumococcal neuraminidase A) removes the terminal sialic acid. The Arachis hypogea lectin specifically recognizes the residual disaccharide β-D-galactose (1-3)-N-acetyl-D-galactosamine (Thomsen-Friedenreich antigen) that is O-glycosidically linked to the serine/threonine residue of glycophorin A (Used with permission of author and Elsevier from Loirat et al. [244]; Copyrighted by Martin Bitzan)

titrate circulating neuraminidase activity by exposing control RBCs to serially diluted plasma from patients with pnHUS [680].

Klein et al. postulated that the interaction of preformed anti-TF antibodies with the exposed neoantigen induces hemolysis, platelet agglutination, microvascular thrombosis, and tissue injury [5]. However, anti-TF antibodies are generally of the IgM class and of low affinity at body temperature [672, 681, 682]. Furthermore, desialylation of RBCs is not specific for HUS: it can be found in patients with IPD without progression to HUS [680, 683, 684], and pnHUS can develop in the absence of TF antibodies [678].

Classification of pnHUS

A practical classification of pnHUS is shown in Table 26.6. It is based on previous publications [665, 668] and refers to coagulation test results at the time of diagnosis, in addition to Coombs test and TF antigen detection. Since other pathogens capable of neuraminidase production, such as *Clostridium perfringens* and influenza A virus, have been identified as causes of HUS [679, 685–689], we suggest the term "pnHUS" for "pneumococcal/neuraminidase (associated) HUS" and distinguish it from "atypical" and other "non-enteropathic" forms of HUS [348, 663, 690].

Presentation of pnHUS and Clinical Course

Patients with pnHUS typically (70–80 %) present with fever and respiratory distress due to lobar pneumonia (70–80 %) that is complicated in two thirds of patients by pleural effusion or empyema [661, 691]. The remaining 20–30 % evolve during bacterial (pneumococcal) meningitis, acute otitis media [660] or pneumococcal sepsis. In one series, 12 % of patients presented with pneumonia and proven or suspected meningitis [661]. The majority (80 %) is bacteremic at the time of diagnosis [692]. The interval between onset of illness attributable to *S. pneumoniae* and the

manifestation of HUS is 1–2 weeks; oliguria develops within 2 weeks of IPD onset [683].

The disease course can be severe or even fatal. A large proportion of patients is admitted to the intensive care unit, of whom >50% require mechanical ventilation and chest tube placement. About 70–85% of patients become oliguric or anuric, often with rapid clinical deterioration, and need acute dialysis [661, 691, 692]. Median time of dialysis in the largest reported series was 10 days (range 2–240 days) [661].

In addition to evidence of microangiopathic hemolytic anemia with profound thrombocytopenia, the laboratory profile is characterized by a rapid rise of acute phase reactants in plasma (CRP, procalcitonin) and an elevated white blood cell count with neutrophilia; however, leucocytopenia may be found in a third of patients [683]. Elevated liver and pancreatic enzymes (amylase/lipase) indicate additional organ involvement. The direct Coombs is positive in 58–90% of patients during the early phase of pnHUS [673, 693–695].

Critically ill patients may present features of HUS and of disseminated intravascular coagulation (DIC) [673, 694, 696, 697]. *S. pneumoniae* sepsis with (mild) anemia, thrombocytopenia, DIC, hypotension and acute kidney injury can masquerade as HUS. Furthermore, Coombs-test positive hemolytic anemia may occur without thrombocytopenia and apparent kidney injury [679, 680]. Investigators therefore felt a need for the definition and classification pnHUS [665, 668, 673] (Table 26.6).

Laboratory Studies and Biomarkers

The defining criterion and most important diagnostic result is the detection of *S. pneumoniae* in physiologically sterile fluids (blood, pleural effusion, CSF, middle ear aspirate, etc.). In case of preceding antibiotic therapy, PCR for pneumococcal-specific nucleic acid sequences or pneumococcal antigen detection should be attempted using pleural fluid, CSF and/or urine.

Laboratory workup of patients with suspected or proven HUS due to neuraminidase-producing organisms should include demonstration of TF exposure and direct Coombs test, in addition to

Table 26.6 Diagnostic criteria for pnHUS

pnHUS	Criteria		Details
Definite	1	Evidence of HUS	Intravascular hemolytic anemia, thrombocytopenia and acute kidney injury (AKI)
	2	Evidence of invasive *S. pneumoniae* infection	Pneumococcal growth/antigen detection or positive PCR from physiologically sterile biological fluid
	3	No evidence of disseminated intravascular coagulation (DIC)	Fibrinogen consumption, prolonged prothrombin (PT) or partial thromboplastin time (PTT), and/or d-dimers *at the time of diagnosis*
Probable	1	Evidence of HUS	See above
	2	Evidence of invasive *S. pneumoniae* infection	See above
	3	(a) Evidence of DIC ***and***	Usually cold agglutinins; TF (Thomsen-Friedenreich) antigen detection by *Arachis hypogaea* or specific lectin/monoclonal antibody binding [679]
		(b) Positive Coombs test and/or evidence of TF antigen exposure	
Possible	1	Evidence of HUS	See above
	2	Suspected (unproven) invasive *S. pneumoniae* infection	Negative culture/antigen detection or PCR from sterile fluid
	3	(a) No evidence of DIC, ***or***	
		(b) Positive Coombs test and/or TF antigen exposure	With or without evidence of DIC (see above)

Data in Copelovitch and Kaplan [668]; and in Spinale et al. [673]

routine coagulation tests, fibrinogen, and d-dimers. Recommended are also C3, C4, CH50, and serum immunoglobulins to exclude congenital or acquired immune deficiencies. Serial CRP measurements, if available, are useful to monitor effective antimicrobial therapy.

No other form of HUS presents with positive Coombs test or TF antigen exposure. However, the frequency of Coombs test positivity in IPD (without evidence of HUS) is not known [673]. Sensitivity and specificity of TF antigen detection was reported as 86 % and 57 %, respectively, for pnHUS or isolated hemolytic anemia. The positive predictive value was 76 %. Conversely, in children with IPD, positive and negative predictive values of TF antigen detection for pnHUS were 52 and 100 % [684].

Complement and pnHUS

Informative studies of the complement system in pnHUS patients are scarce. In one series, two of five previously healthy children with pnHUS were found to carry known, heterozygous mutations in *CFI* and *CFH* and one had a possibly damaging variant in the gene coding for thrombomodulin (*THBD*); three patients had a CFH related protein one third (*CFHR1/3*) deletion (two in combination with a *CFH* or *CFI* mutation), but none had detectable anti-CFH antibodies. All five patients were Coombs positive and demonstrated mild to moderate depression of serum C3 and C4. ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) activity was preserved [698]. Another group reported two patients with pnHUS, both with transiently depressed serum C3, but normal C4 concentrations, normal ADAMTS13 activity and no detectable mutations in the studied alternative pathway of complement regulator genes [699].

Complement consumption, primarily due to activation of the alternative pathway, appears to be a frequent, but not obligatory event in patients with pnHUS. However, *S. pneumoniae* infection can trigger HUS in children with damaging mutations of complement regulator genes, similar to non-specific agents in patients with "atypical" HUS. Interestingly, disease relapses due to *S. pneumoniae* have not been described. The emergence of specific immunity is surmised, not only to the pneumococcal serotype but common virulence factors.

Treatment of pnHUS

Treatment of patients with pnHUS includes appropriate antibiotics and best supportive care [244]. As with eHUS, symptomatic anemia, significant bleeding or surgery are indications for PRBC and platelet transfusions, respectively. Dialysis (hemodialysis or peritoneal dialysis, depending on local expertise and preference) is required in up to 80 % of patients. Some patients with *S. pneumoniae* sepsis or sepsis due to a secondary organisms and hemodynamic instability may benefit from CRRT.

Previous recommendations to only transfuse "washed" RBCs and to avoid administration of plasma (which contains anti-TF antibodies) – or to select plasma with low anti-TF titers [700, 701] – are not based on evidence [14, 244, 662, 673]. Indeed, plasma therapy (plasma infusion or plasma exchange) has been described in pnHUS patients without apparent worsening of hemolysis or renal function [661, 702, 703].

Although reported, currently available data do not suggest that patients with pnHUS should be subjected to therapeutic complement blockade, unless there is a proven or suspected complement regulator defect [699].

Outcome

Unfavorable outcome is expected in about 20 % of patients in the largest case series comprising 43 patients from the UK in 2007 [661]. From a recent North American series, 10 % of patients with ≥6 months follow-up had undergone kidney transplantation, 13 % had neurologic sequelae, and 3 % had died [692]. Important CNS complications are intracranial hemorrhage and

infarction, leading to obstructive hydrocephalus in some patients and sensorineural hearing loss [661, 662]. Waters et al. reported that only 2 of 13 patients with pnHUS and meningitis demonstrated a normal neurodevelopmental outcome [661]. Pulmonary complications in addition to empyema include pneumatoceles and necrotic pneumonic changes [662].

Investigators from France, the UK and New Zealand noted mortality rates of 27 %, 11 % and 9 %, respectively [661, 662, 704]. In contrast, no fatalities were recorded in patients with *S. pneumoniae*-related HUS during the SYNSORB Pk^R clinical trial [663]. There is substantial variability of the reported mortality rates (0–13 %), which is best explained by the small number of cases even in the largest reported series [673, 690]. Most deaths are not caused directly by HUS or renal injury, but are related to complicating pneumococcal meningitis and sepsis/shock.

Although the clinical course tends to be more severe in HUS following *S. pneumoniae* than STEC infection, as indicated by frequency of PRBC transfusions and dialysis [661, 691, 704], the long-term renal outcome in surviving patients does not appear to be worse than in patients with STEC-HUS [663, 690]. Residual renal dysfunction, reflected by decreased GFR and the presence of proteinuria, has been variably reported in 20–25 % [661, 690]. Kidney transplantation following pnHUS is very rare, and experience is limited [705, 706].

Influenza HUS

Epidemiology and Importance

Few proven cases of HUS triggered by influenza virus infection (iHUS) have been published. In all instances with appropriate viral diagnostic, HUS was associated with influenza A strains, mainly A(H3N2) and A(H1N1) (Table 26.7). The first retrievable description is from a 20 year-old kidney transplant recipient, from 1971 [707]. The patient was diagnosed with microangiopathic hemolytic anemia and graft failure 1–2 weeks

Table 26.7 Demographic and clinical data of influenza HUS

Demographic and clinical features	Details	Median (range), frequencies[a]
Age (years)		14.5 (3–34) years, n = 12
Influenza strains	A(H3N2)	2
	A(H1N1)	7
	A (serology only)	3
Renal status	Native kidneys	9/12 (75 %)
	Kidney allografts [707–709]	3/12 (25 %)
Hematology	Hemoglobin (nadir)	78 (50–111) g/L (n = 11)[a]
	Platelets (nadir)	29 (8–80) × 10^9/L (n = 12)
	LDH (peak)	2888 (300–13,188) U/L (n = 7)
	Positive Coombs test	None (n = 8)
AKI	Serum creatinine (peak)	309 (230–701) µmol/L (n = 10)
Complement	Low C3	3/8 (27 %)[a,b]
	Low C4	None (n = 5)[a,b]
	Complement regulator deficiency or relapsing HUS[b]	2
Treatment	Dialysis	7/12 (58 %)
	Plasma infusion	4/12 (33 %)
	Plasma exchange	3/12 (25 %)
	Eculizumab	1/12 (8 %)
Outcome	Complete recovery	10/12 (83 %)
	CKD	1/11 (9 %)[c]
	Death	1/12 (8 %)
	Graft loss	1/3 (33 %)

Data from References [688, 689, 707–715]

[a]Number of patients with reported results. Three additional cases, not included in this table, have been reported as TTP (see text) [716–718]

[b]Mutation of C3 (Transplant recipient); iHUS in third graft [709]; relapsing HUS (native kidney) without identified complement mutation; fourth episode triggered by influenza A/H1N1 infection [715]; decreased C3 (complement mutation studies not reported) [713]

[c]Graft loss (transplant recipient); deceased patient excluded

after the onset of influenza, almost 2 years after transplantation; additional laboratory features included cold agglutinins (with negative direct Coombs test) and transiently reduced plasma C3 concentration. A graft biopsy 5 weeks after HUS onset revealed thrombosis of small renal arteries and glomerular capillaries. The graft was removed 8 weeks after HUS onset, followed by swift normalization of the hematological parameters. A subsequent graft from a deceased donor (DD) was tolerated well without recurrence of HUS.

Several cases of iHUS were noted during the pandemic influenza A(H1N1). A typical scenario is that of a previously healthy, 7-year-old boy with febrile pneumonitis and transient respiratory failure who developed severe AKI, profound microangiopathic hemolytic anemia and thrombocytopenia associated with hypertensive encephalopathy 5 days after the onset of respiratory symptoms. Coagulation profile, plasma fibrinogen, Coombs test and C3 concentration were normal as was the screening for MCP expression, plasma ADAMTS13 activity and CFB, CFH and CFI concentrations. He recovered completely after 2 weeks of peritoneal dialysis [710]. Additional patients with influenza A-associated HUS demonstrated elevated d-dimers [688, 711, 712]. None of the tested patients had a positive Coombs test [689, 707, 708, 710, 713, 714], but cold agglutinins were reported once [707]. It is currently unclear if A(H1N1) has a greater propensity to induce HUS than other influenza strains.

Pathogenesis

There is an established link between influenza virus infection and HUS, but the mechanism that triggers iHUS remains speculative [13]. Influenza virus shares with *S. pneumoniae* the ability to express neuraminidase (NA). Hemagglutinin (HA) and NA are defining and important (viral) pathogenicity factors in human (and animal) infections. NA shedding in influenza infection is minimal compared to *S. pneumoniae*, and it remains to be shown if it contributes to the pathogenesis HUS.

Influenza A virus attachment to sialic acid residues on host (target) cells is mediated by viral surface-exposed HA. Once endocytosed, virus redirects the host cell machinery to serve its replication and turns host cell RNA transcription and translation off. NA is responsible for virion release and propagation of the infection through the cleavage of sialic acid residues on host cells [719]. Autopsy studies during the 2009 A(H1N1) pandemic showed viral antigen in endothelial cells [720, 721]. *In vitro* infection of endothelial cell by influenza virus [722] can trigger apoptosis [723], a process known to stimulate platelet adhesion directly and via the exposure of extracellular matrix [289, 724].

In addition to injuring or activating vascular endothelial cells, influenza virus may directly affect platelets. A(H3N2) virus induces clumping of human and rabbit platelets *in vitro* and a rapid drop of platelet counts *in vivo* after injection of the virus into rabbits [725]. More recent studies confirmed the potential of influenza virus to activate platelets and generate thrombin, among others [726, 727]. In a prospective study comparing patients with ARDS due to severe influenza A(H1N1) and bacterial pneumonia with healthy controls, influenza showed the greatest degree of platelet activation measured as formation of platelet-monocyte aggregates and activation of αIIbβ3 integrin on platelets [726].

Influenza-Associated HUS and Complement Dysregulation

Plasma C3 levels have been reported in eight patients, based on a review of the accessible literature; they were reduced in three of eight cases. Three patients were found (or suspected) to have genetic complement regulator defects: a 15 year-old boy with a gain-of-function mutation in the C3 locus, who had lost two previous kidney allografts due to HUS recurrences (treated with eculizumab) [709], a 17 year-old boy with reduced plasma C3 and heterozygous CD46 deficiency (second episode of HUS) [714] and a 15 year-old girl with incomplete genetic workup (normal C3 and C4, factor H, and factor I levels,

undetectable CFH autoantibody, and lack of *CHF* mutation), who had four preceding spontaneously resolving episodes of HUS (Table 26.7) [715]. In these instances, HUS should be viewed as "atypical," triggered by influenza A infection. The remaining nine patients, including a transplant recipient with HUS, had no preceding episodes of HUS. Thus, the majority of the reported cases iHUS appear to occur spontaneously, without known complement regulator defect. However, only two of the reported patients have been screened for alternative pathway regulator abnormalities. The question, whether genetic (host) factors confer susceptibility and whether complement plays a role in iHUS warrants further studies.

Influenza and Streptococcus Pneumoniae Infection

Influenza virus infections are known to increase the susceptibility of the host to the propagation of *S. pneumonia* [728]. Co-infection can pose a challenge determining which of the pathogens is responsible for pulmonary complications and HUS [13].

Influenza and TTP

Influenza A virus infections, including A(H1N1) have been invoked as a cause of TTP in at least three published cases [716–718]. TTP is defined by nearly absent (<10 %) ADAMTS13 activity [729]. Kosugi [716] reported a 68 year-old patient with influenza A infection–associated TTP with <0.5 % ADAMTS13 activity and increased inhibitor concentration. The TTP diagnosis of the remaining two cases was based on the presence of neurological manifestations, such as headache and mental confusion [718] or hemiplegia [717]. However, neither ADAMTS13 activities or anti-ADAMTS13 antibody measurements nor complement genetic studies were reported in the latter cases. It therefore remains to be shown if influenza virus can induce TTP, based on current definitions (lack of active ADAMTS13) or whether these were cases of HUS with CNS manifestations.

Laboratory Diagnosis

All patients with HUS due to infections by seasonal or epidemic influenza strains should undergo rapid testing for plasma C3 and, if available, SC5b-9 concentrations, and ADAMTS13 activity and autoantibodies. TTP is suspected in patients with minimal or no renal injury [553, 729]. The presence of concomitant or complicating pneumococcal pneumonia or sepsis must be ruled out in any case of (suspected) iHUS (see above).

Therapeutic Management

Best supportive care includes judicious transfusion of RBCs and platelets, dialysis and other supportive measures. It is unknown if the NA inhibitor oseltamivir prevents or ameliorates iHUS. Where its administration has been reported, it has been given at or after the onset of HUS [713, 714]. The role of plasma therapy (PI, PLEX) or of eculizumab in iHUS is unproven. However, recommendations for the treatment of "atypical" HUS should be followed if the patient presents evidence of complement dysregulation, i.e., known complement gene mutation or CFH antibody, preceding HUS episode(s) or positive family history of (a) HUS, or recurrence of HUS after kidney transplantation [12, 244, 348].

HIV HUS

Epidemiology and Clinical Presentation

TMA in the context of AIDS has been variably described as HUS or TTP. In fact, TTP has been listed as an AIDS defining condition [730, 731]. However, AIDS-related infections or complications can present clinical features that resemble TMA [732].

One of the first reports of TTP in a patient with AIDS appeared in 1984 [733]. In a large series by Moore et al. [732], 350 consecutive, hospitalized adult patients with AIDS in the mid-1990s were evaluated for the presence of a TTP-like syndrome, i.e., anemia, thrombocytopenia, fragmented erythrocytes, renal and neurologic dysfunction, and fever [732]. Schistocytosis was present in 24 %, and the full clinical picture of TTP in 7 % of the patients. Patients with TMA were more likely to have a low CD4 lymphocyte count, CDC stage C disease, and bacterial sepsis [732].

Based on the number of published reports, the incidence of HIV TMA has decreased since the advent of ART and HAART therapies [6]. A study from the Oklahoma TTP-HUS Registry covering an 18-year period, from 1989 to 2007 [7], found evidence of HIV infection in 6 of 326 patients with a diagnosis of TTP (1.84 %; $CI_{95\%}$ 0.68 %–4.01 %). The authors calculated a period prevalence for 1989–2007 of HIV infection among all adults in the Oklahoma TTP-HUS Registry region of 0.30 %. One patient had multiple relapses. However, there was a large overlap between TTP-like conditions and other AIDS-related complications [7].

Pathogenesis

Measurements of ADAMTS13 activity or antibodies, of complement activation, anti-CFH antibodies, or the presence of neuraminidase/NA-mediated desialylation (TF antigen) have not been studied. The direct Coombs test appears to be negative [733]. None of the described children presented with bloody diarrhea or evidence of STEC O157:H7 infection. The evolution of the HUS and its severity are variable.

Clinical observation and animal experimentation suggest that HIV can directly cause HUS [734]. The mechanism(s) leading to HUS remain unclear. HIV infects glomerular endothelial and mesangial but not epithelial cells *in vitro* [735]. Intriguingly, a viral surface glycoprotein, pg120, binds to Gb3, the Stx receptor; this interaction has been linked with the occurrence of HIV HUS [215, 734, 736]. Conversely, Gb3 has been described as natural resistance factor for the prevention of HIV infection, e.g., when given as soluble agent [737, 738].

ADAMTS13 deficiency, characteristic for TTP [739], has rarely been reported in patients with HIV TMA. Some authors noted difficulties in the interpretation of ADAMTS13 activity levels which may develop after repeat episodes or independent of clinical signs of TMA, leading to the conclusion that measurement of ADAMTS13 activity cannot distinguish patients with "typical TTP" from those where TMA is subsequently attributed to another etiology [7, 740].

Clinical Presentation and Outcome

Clinical information of HIV HUS or HUS-like conditions is limited to a few anecdotal reports and summary statistics. An instructive case is that of a 12-year-old boy with transfusion-associated HIV infection [8]. He presented with fever, abdominal pain, and cough, 8 months after the detection of HIV infection. The absolute CD4 count was 10 cells/mm^3. HUS was diagnosed based on the presence of (severe) intravascular hemolysis, moderate thrombocytopenia, and gradually rising serum creatinine with mild oliguria. There was no infectious focus. Stool cultures failed to grow common enteropathogens, and *E. coli* O157 LPS serology was non-diagnostic. Blood and urine cultures were negative. Both kidneys appeared echogenic by ultrasound.

Treatment consisted of daily infusions of frozen plasma, 10 ml/kg per dose over 10 days. Hemolysis and the need for PRBC infusions diminished 2 weeks after the first plasma infusion. The platelet count increased a week after discontinuation of plasma infusions. However, he became dialysis-dependent and subsequently died after treatment withdrawal [8].

The second case was that of a 6-month old infant. She too experienced gradual loss of renal function despite plasma infusions for 10 days. She succumbed to unstoppable gastrointestinal bleeding 17 days after diagnosis [8].

A third report describes a previously undiagnosed adult male with HIV infection presenting with fever, nausea, diarrhea and reduced urinary output [6]. HUS was diagnosed and treatment with plasmapheresis and hemodialysis started. Hb, platelets, LDH and renal function normalized after four daily treatment sessions.

Renal Pathology

The kidney biopsy of the first case of pediatric HIV described above [8], revealed marked thickening of the walls of glomerular capillaries, arterioles and small arteries, vacuolar endothelial changes, luminal thrombosis and presence of intravascular red cell fragments. There was focal tubular atrophy, interstitial fibrosis, and a mild focal mononuclear interstitial infiltrate composed, predominantly, of lymphocytes and a few plasma cells. Immunofluorescent staining for IgG and IgM was negative, but there was 1+ focal staining of the capillary walls for fibrinogen and C3. Electron microscopy showed no electron-dense deposits.

Therapeutic Management of HIV TMA

Some authors found HIV TTP to be highly responsive to PLEX therapy [741]. Given the overall limited evidence, others suggested cautious consideration of plasma exchange for HUS or TTP in AIDS patients [6–8]. There are no guidelines for children with TMA-like disorders associated with HIV infection. Effective treatment of the HIV infection is expected to help recovery from HIV-associated TMA. Careful evaluation is recommended to exclude alternative diagnoses, such as malignant hypertension or disseminated Kaposi sarcoma [7].

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