Red blood cell Thomsen-Friedenreich antigen expression and galectin-3 plasma concentrations in *Streptococcus pneumoniae*–associated hemolytic uremic syndrome and hemolytic anemia

Nicolas Burin des Roziers,[1] Philippe Chadebech,[1,2] Gwellaouen Bodivit,[1,2] Emmanuelle Guinchard,[3] Arnaud Bruneel,[4] Thierry Dupré,[4] Laurent Chevret,[5] Myriam Jugie,[6] Philippe Gallon,[1] Philippe Bierling,[1,2] and France Noizat-Pirenne[1,2]

BACKGROUND: Pneumococcal hemolytic uremic syndrome (P-HUS) is a rare but severe complication of invasive pneumococcal disease (IPD) in young children. Consensual biologic diagnosis criteria are currently lacking.

STUDY DESIGN AND METHODS: A prospective study was conducted on 10 children with culture-confirmed IPD. Five presented with full-blown P-HUS, three had an incomplete form with hemolytic anemia and mild or no uremia (P-HA), and two had neither HUS nor HA. Thomsen-Friedenreich (T), Th, and Tk cryptantigens and sialic acid expression were determined on red blood cells (RBCs) with peanut (PNA), *Glycine soja* (SBA), *Bandeiraea simplicifolia II*, and *Maackia amurensis* lectins. Plasma concentrations of the major endogenous T-antigen–binding protein, galectin-3 (Gal-3), were analyzed.

RESULTS: We found that RBCs strongly reacted with PNA and SBA lectins in all P-HUS and P-HA patients. Three P-HUS and three P-HA patients showed also concomitant Tk activation. Direct antiglobulin test (DAT) was positive in three P-HUS (one with anti-C3d and two with anti-IgG) and two P-HA patients (one with anti-C3d and one with anti-IgG). RBCs derived from the two uncomplicated IPD patients reacted with PNA but not with SBA lectin. Gal-3 plasma concentrations were increased in all P-HUS patients.

CONCLUSIONS: The results indicate high levels of neuraminidase activity and desialylation in both P-HUS and P-HA patients. T-antigen activation is more sensitive than DAT for P-HUS diagnosis. Combining PNA and SBA lectins is needed to improve the specificity of T-antigen activation. High concentrations of Gal-3 in P-HUS patients suggest that Gal-3 may contribute to the pathogenesis of P-HUS.

Hemolytic uremic syndrome (HUS) is characterized by acute microangiopathic hemolytic anemia (HA), thrombocytopenia, and acute kidney injury. Most cases of childhood HUS follow an episode of diarrheal illness associated with Shiga toxin–producing *Escherichia coli* (STEC).

A distinct and rare form of HUS is associated with invasive *Streptococcus pneumoniae* infections in young children. *S. pneumoniae*–associated HUS (P-HUS)

ABBREVIATONS: 2-DE = two-dimensional electrophoresis; APC = alternative pathway of complement; ASGPR = asialoglycoprotein receptor; BS-II = *Bandeiraea simplicifolia II;* CFH = complement factor H; Gal-3 = galectin-3; HUS = hemolytic uremic syndrome; IPD = invasive pneumococcal disease; MAA = *Maackia amurensis*; P-HA = IPD patients with hemolytic anemia; P-HUS = *Streptococcus pneumoniae*–associated HUS*;* PNA = *Arachis hypogaea* (peanut agglutinin); SBA = *Glycine soja;* STEC = Shiga toxin–producing *Escherichia coli*; Trf = transferrin.

From the [1]Etablissement Français du Sang Ile de France, Villejuif, France; [2]Inserm UMR955, Créteil, France; the [3]Etablissement Français du Sang Rhône-Alpes, Lyon, France; the [4]Laboratoire de Biochimie Métabolique et Cellulaire, Hôpital Bichat, Paris, France; the [5]Réanimation Pédiatrique, Hôpital de Bicêtre, Le Kremlin Bicêtre, France; and the [6]Réanimation Chirurgicale Pédiatrique, Hôpital Necker-Enfants Malades, Paris, France.

Address reprint requests to: Nicolas Burin des Roziers, MD, Etablissement Français du Sang Ile de France, Hôpital Paul Brousse, 14 Avenue Paul Vaillant-Couturier, 94800, Villejuif, France; e-mail: nicolas.burin-des-roziers@efs.sante.fr.

Received for publication July 15, 2014; revision received October 30, 2014, and accepted November 7, 2014.

doi: 10.1111/trf.12981

© 2014 AABB

TRANSFUSION 2015;55:1563–1571

accounts for 5% of cases of childhood HUS and nearly 40% of cases not associated with STEC, with an observed increase in prevalence in recent years.[1,2] P-HUS is more severe than HUS caused by STEC with a mortality rate between 2 and 12%, according to the underlying clinical condition, and end-stage renal disease occurring in 10% to 16% of survivors.[2]

Pneumococcal neuraminidase activity is usually considered to be the major trigger of P-HUS.[1-3] Neuraminidase released by *S. pneumoniae* acts by cleaving terminal *N*-acetylneuraminic acid (sialic acid) from glycoproteins present in plasma and on cell surfaces in contact with the enzyme and exposing the "cryptic," normally hidden by sialic acid, ubiquitous Thomsen-Friedenreich antigen (T-antigen). This process is known as T-activation.[4,5] Three neuraminidases are encoded by the *S. pneumoniae* genome, NanA, NanB, and NanC. NanA is necessary for significant neuraminidase activity in vitro[6] and for T-activation during pneumococcal infection in mouse models.[3] The nanA gene is present in 100% of *S. pneumoniae* strains. Nevertheless, only a small percentage of invasive pneumococcal disease (IPD) patients progress to P-HUS. The incidence of P-HUS is estimated to be 0.4% to 0.6% of all IPD.[1,2]

Little is known about the relationship between the level of neuraminidase activity and the risk of developing HUS in IPD. In vitro the occurrence of P-HUS is not correlated with the expression of nanA and nanB genes, the nanA allelic diversity, and the neuraminidase enzymatic activity of *S. pneumoniae* isolates.[6] To date, the best evidence for a role of neuraminidase in the pathogenesis of P-HUS is supported by the study of Huang and colleagues[7] who showed that T-activation is significantly associated with HUS in IPD. Using peanut agglutinin (PNA; *Arachis hypogaea*), the authors detected T-antigen in 13 of 13 children with P-HUS and in six of 14 children with uncomplicated invasive pneumococcal infections. Thus, T-activation was 100% sensitive for P-HUS diagnosis and 57% specific. However, PNA may react with two other cryptantigens frequently exposed by pneumococcal exoglycosidases, Th and Tk.[5,8] Quantitative studies or additional lectins need to be used to assess the real specificity of T-activation for P-HUS diagnosis.

The mechanisms by which neuraminidase or T-antigen contribute to the pathogenesis of P-HUS are still not understood.[1-3,6] Anti-T immunoglobulin (Ig)M naturally occurring antibodies are present in most normal human sera.[5,9] Therefore, since early reports,[10] endothelial injury, hemolysis, and P-HUS were assumed to result from the interaction of preexisting anti-T with T-antigen exposed by neuraminidase on glomerular endothelial cells and red blood cells (RBCs).[2,10] However, the characteristics of anti-T antibodies cast doubts on their pathogenic role in endothelial damage. We showed previously that anti-T are low-titer antibodies with extremely weak hemolyzing capacities compared with ABO antibodies.[9] Furthermore, anti-T have never been definitely shown to cause hemolysis in patients with T-activated RBCs.[4,5,11] Finally, P-HUS may occur in children aged 4 or 5 months,[12-14] an age where a significant amount of anti-T is unlikely to be present.

Recently, new hypotheses have been suggested to explain the pathogenesis of P-HUS, based on transient dysregulation of the complement activation.[6,14,15] Besides its role in demasking T-antigen, desialylation is also known to trigger alternative pathway of complement (APC) dysfunction on RBCs. The major soluble regulator of APC, Factor H, binds to sialic acids. Factor H functions as a cofactor for the Factor I–mediated C3b cleavage. It has been shown that desialylated RBCs activate the complement system via the APC in vitro[16] and also in vivo, as demonstrated in guinea pigs.[17]

Endogenous ligands of T-antigen include the asialoglycoprotein receptor (ASGPR) of hepatocytes, involved in clearance of asialoglycoproteins, and galectins, a family of lectins, characterized by their affinity for β-galactoside–containing oligosaccharides. Desialylated platelets (PLTs) exposing β-galactose residues are rapidly cleared from blood circulation by the ASGPR.[18,19] By contrast with PLTs, the ASGPR plays no role in removal of desialylated RBCs. Complement activation is required for the clearance of desialylated RBCs.[17]

On the other hand, soluble proteins known as galectins are characterized by their affinity for T-antigen. Among galectins, galectin-3 (Gal-3) has the highest affinity to T-antigen.[20]

Gal-3 is expressed and secreted by macrophages and other immune and nonimmune cells[21] and is implicated in several aspects of the inflammatory response, cell migration, cell adhesion to endothelium,[21-23] and host defense against pneumococcal infection.[24] Gal-3 oligomerizes at high concentrations and forms heterogenous multimolecular complexes that cross-link both cell surfaces and glycoconjugates expressing the T-antigen.[25] Furthermore, T-antigen/Gal-3–mediated cell interactions with endothelium are stabilized by endothelial integrins that associate directly with T-antigen/Gal-3 complexes.[26]

Taken together, this information led us to hypothesize that adhesion between desialylated RBCs and endothelial cells, both expressing T-antigen, may occur in the presence of high concentrations of Gal-3. This adhesion could generate complement activation in close contact with the endothelial cell layer, promoting endothelial injury and HUS.

In this study, we investigated T-antigen and sialic acid expression on RBCs and Gal-3 plasma concentrations in a series of 10 patients refered to our laboratory for the diagnosis of P-HUS. In addition, we carried out direct antiglobulin test (DAT) in these patients and compared DAT results with T-antigen testing for the biologic diagnosis of P-HUS.

15372995, 2015, 6pt2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/trf.12981 by Arnaud Brunel - Assistance Publique Hopitaux De Paris Ap-Hp , Wiley Online Library on [20/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

MATERIALS AND METHODS

Patients

Blood samples from 10 consecutive patients with a diagnosis of IPD (pneumococcal pneumonia or pneumococcal meningitis) were prospectively included between May 2010 and December 2013. We included only patients for whom samples taken before the first transfusion were available. Blood samples from pediatric departments of two hospitals were sent to our laboratory for the diagnosis of T-antigen activation. The medical records of the 10 IPD patients were retrospectively reviewed. The criteria for diagnosis of HUS were microangiopathic HA (hemoglobin [Hb] <10g/dL with fragmented RBCs on blood smear), thrombocytopenia (platelet [PLT] count < 130×10^9/L), and acute renal impairment (>88.4 µmol/L or >50% increase above baseline). Disseminated intravascular coagulation was excluded by the presence of normal fibrinogen concentrations at the time of HUS diagnosis.

Clinical features and laboratory findings of IPD patients are summarized in Table 1. A control group of 238 pediatric patients hospitalized in a neonatal intensive care unit was also tested for RBC T-activation to assess the specificity of the lectins used in the study.

Because T-activation is commonly detected in infants with necrotizing enterocolitis,[4,11] necrotizing enterocolitis patients were excluded from this control group. Screening was carried out on residual samples of fresh EDTA-anticoagulated peripheral blood residual samples remaining in the hematology laboratory after routine blood analysis. The blood samples were destroyed thereafter.

Lectins

PNA lectin agglutinates T, Th, and Tk transformed RBCs.[5,8] Specific lectins were used in this study to differentiate T and Tk cryptantigens and the attenuated form of T activation called Th (Table 2). PNA and *Glycine soja* (soybean, SBA) lectins were purchased from Sigma Aldrich (Sigma Chemical Co., St Louis, MO) and *Bandeiraea simplicifolia II* (BS-II) lectin was purchased from Vector Laboratories (Burlingame, CA). The expression of sialic acid on RBCs was investigated with *Maackia amurensis* (MAA) lectin obtained from Sigma Aldrich. Lectins were diluted in 0.01 mol/L phosphate-buffered saline (pH 7.4; Sigma Aldrich) to obtain a concentration of 0.2 mg/mL (MAA), 0.5 mg/mL (BS-II and SBA), or 0.025 mg/mL (PNA). These samples were then aliquoted and stored at –30°C until use. The same primary stock for each lectin was used throughout the study.

Agglutination tests

Agglutination tests were carried out by the standard gel technique using microtubes containing neutral gel

TABLE 1. Clinical and laboratory characteristics of patients with invasive pneumococcal diseases

Patient	Sex/age (months)	Infection	*S. pneumoniae* serotype	HUS/HA	Creatinine peak (µmol/L)	Dialysis	Minimal Hb (g/dL)	Schizocytes	Minimal PLT count ($\times 10^9$/L)	Blood transfusions	Outcome
1	Female/7	Meningitis	16F	HUS	ND	Yes	6.3	Yes	111	RBC (B), PC (W)	Died
2	Female/21	Pneumonia	ND	HUS	370	Yes	6.0	Yes	15	RBC (UW), PC (UW), FFP (PE)	Recovery
3	Male/18	Meningitis	6C	HUS	272	Yes	5.2	Yes	26	RBC (B)	Recovery
4	Male/12	Meningitis	15A	HUS	240	No	7.3	Yes	11	RBC (UW), PC (UW)	Died
5	Female/6	Meningitis	1	HUS	118	Yes	6.5	Yes	29	RBC (UW), PC (UW)	Recovery
6	Male/13	Meningitis	15A	HA	88	No	6.6	No	92	RBC (W)	Recovery
7	Male/7	Meningitis	ND	HA	60	No	6.0	No	21	RBC (UW)	Died
8	Female/11	Meningitis	15B	HA	18	No	6.1	Yes	19	RBC (W), PC (UW)	Recovery
9	Female/4	Meningitis	ND		21	No	7.1	No	420	RBC (UW)	Recovery
10	Male/11	Meningitis	24F		18	No	10.2	No	553	RBC (UW)	Recovery

B = both washed and unwashed blood products; FFP = fresh-frozen plasma; ND = not determined; PC = PLT concentrate; PE = plasma exchange; UW = unwashed; W = washed.

15372995, 2015, 6pt2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/trf.12981 by Arnaud Brunel - Assistance Publique Hopitaux De Paris Ap-Hp , Wiley Online Library on [20/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2. Interaction of T, Th, and Tk transformed RBCs with selected lectins

Lectin	Combining carbohydrate	T	Th	Tk
PNA	Galβ1,3GalNAc	+	+	+
SBA	GalNAc	+	–	–
BS-II	GlcNAc	–	–	+
MAA	NeuAcα2,3Gal	–*	+	+

* MAA may react with T when weakly expressed.

TABLE 3. Agglutination by lectins of untreated and neuraminidase-treated RBCs

Neuraminidase (U/mL)	PNA (agglutination titer)	MAA (agglutination titer)
0	<1	64
0.1	1	64
1	128	8
10	128	1
100	128	<1

(DiaMed, Cressier, Switzerland). A total of 50 μL of 0.8% RBCs was incubated with 25 μL of diluted lectins for 30 minutes at 22°C. Thereafter, the gel cards were centrifuged for 10 minutes at 85 × *g*. Agglutination results were scored according to DiaMed specifications. Titers were the highest dilution resulting in hemagglutination. At least two independent experiments were carried out for each titration. Positive and negative controls were included in each series of titrations. Positive controls were RBCs from volunteer blood donors treated with various concentrations of *Vibrio cholerae* neuraminidase (Sigma Aldrich, N6514) as described previously,[9] and negative controls were untreated RBCs from the same donors. Representative results obtained with positive and negative controls are given in Table 3.

DAT

A DAT was performed by the gel technique using microtube gel cards containing anti-IgG (rabbit) and anti-C3d (monoclonal) reagents (DC Screening Cards, DiaMed). A total of 50 μL of 0.8% RBC suspensions in low-ionic-strength solution were added to the microtubes. Gel cards were centrifuged for 10 minutes at 85 × *g*. The DAT positive results were scored from 1+ to 4+ as indicated by DiaMed. Because samples were very small, no elution procedure was performed on DAT-positive RBCs.

Two-dimensional electrophoresis

Two-dimensional electrophoresis (2-DE) coupled with Western blotting was performed as described previously.[27] Proteins from 1 μL of plasma were separated by 2-DE using the ZOOM strip pH 4-7 system for the first dimension and ready-made 4% to 12% NuPAGE Bis-Tris gels for the second dimension, as recommended by the manufacturer (Life Technologies, Saint Aubin, France). After 2-DE, proteins were transferred (100 V for 1 hr) to nitrocellulose and glycoforms of interest were detected with an anti-transferrin (Trf) rabbit antibody (1/5000 vol/vol from Siemens Healthcare Diagnostics, Marburg, Germany), a horseradish peroxidase–linked anti-rabbit IgG secondary antibody (1/5000 vol/vol; GE Healthcare, Uppsala, Sweden) and an imaging system (Chemidoc XRS, Bio-Rad, Marnes la Coquette, France).

Gal-3 assay

Gal-3 plasma concentrations were assayed with the Gal-3 enzyme-linked immunosorbent assay (ELISA) kit (Calbiochem, Merck Chemicals Ldt, Nottingham, UK) according to the manufacturer's instructions. Normal human blood samples were used to establish the normal range of Gal-3 values and were obtained from volunteer blood donors after informed consent. A standard curve was made with dilutions of a Gal-3 protein standard. All plasma samples were tested in duplicate.

Statistical analyses

Quantitative data are expressed as arithmetic mean ± standard error (SEM); statistical analyses were performed with t tests and the computer software (GraphPad Prism, Version 5.03, GraphPad, Inc., La Jolla, CA). Values of $p < 0.05$ were considered significant.

RESULTS

Diagnosis of HUS

Ten patients with IPD were prospectively included in this study. Nine of them had meningitis. Five patients were classified as definite P-HUS based on criteria from the Center for Disease Control. Disseminated intravascular coagulation was excluded in all cases based on normal fibrinogen concentrations. Four different serotypes of *S. pneumoniae* strains were identified in P-HUS patients: 1, 6C, 15A, and 16F (Table 1). Two other patients with pneumococcal meningitis presented with acute HA, thrombocytopenia, and higher than normal creatinine concentrations (Patient 6, Serotype 15A; and Patient 7). However, these two patients did not fully meet the criteria of HUS because schizocytes were not found on repeat blood smear examinations. Another patient (Patient 8, Serotype 15B) presented with acute microangiopathic HA and thrombocytopenia with normal creatinine concentrations. Two patients with pneumococcal meningitis had no sign of HUS (Patients 9 and 10).

15372995, 2015, 6pt2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/trf.12981 by Arnaud Bruneel - Assistance Publique Hopitaux De Paris Ap-Hp , Wiley Online Library on [20/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

15372995, 2015, 6pt2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/trf.12981 by Arnaud Brunel - Assistance Publique Hopitaux De Paris Ap-Hp , Wiley Online Library on [20/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4. Results of lectin agglutination tests, DAT, and Gal-3 plasma concentrations of patients with IPD

Patient	HUS/HA	RBC transformation (T/Th)	PNA (agglutination titer)	MAA (agglutination titer)	Tk transformation	DAT	Gal-3 (ng/mL)
1	HUS	T	128	<1	–	1+ IgG	33
2	HUS	T	128	8	+	1+ C3d	27
3	HUS	T	128	64	–	1+ IgG	22
4	HUS	T	128	128	+	–	41
5	HUS	T	128	4	+	–	ND
6	HA	T	128	16	+	2+ IgG	8
7	HA	T	128	64	+	–	26
8	HA	T	128	128	+	3+ C3d	25
9		Th	8	64	–	–	15
10		Th	8	128	–	–	9
Negative controls*			<1	64-128	ND	ND	ND

* Negative controls: RBCs from uninfected newborns.
ND = not determined.

Lectin agglutination of RBCs

RBC T-activation was initially examined in 238 infants from a neonatal intensive care unit to assess the specificity of PNA agglutination method. None of these neonates tested positive for specific T-activation confirmed with SBA lectin. However, one neonate tested positive with PNA (Titer 2) and negative with SBA lectins and was thus identified as Th-activated. The blood culture of this infant was positive for *S. pneumoniae*.

Table 4 shows the results of RBC testing in the acute phase of IPD for the 10 patients included. All tests were performed on pretransfusional samples. T and Tk cryptantigens were frequently expressed on RBCs from these patients. T cryptantigen confirmed with SBA lectin was expressed in 100% of P-HUS and P-HA patients (all five P-HUS and all three P-HA patients). Th activation was found in the two IPD patients with no signs of HUS. Titration with PNA showed that the T-antigen was strongly exposed (agglutination titer 128) in all P-HUS and P-HA patients. By contrast, the agglutination titer with PNA was only 2 for both IPD patients with neither HUS nor HA. Tk transformation was found in six of 10 IPD cases, including three of five HUS patients, and was always associated with T transformation. Analysis of sialic acid on the surface of RBCs with *M. amurensis* lectin showed that sialic acid expression was lower than normal in three of five P-HUS patients and in one of three P-HA patients (Patient 6).

DAT

DAT results are shown in Table 4. Positive DAT results (from 1+ to 3+) were found in three of five P-HUS patients and in two of three P-HA patients. DAT was positive with anti-C3d for two patients (1+ for Patient 2 and 3+ for Patient 8) and with anti-IgG for three other patients (Patients 1, 2, and 6).

2-DE

2-DE of plasma Trf was performed for five of 10 patients (three P-HUS patients, one P-HA patient, and one IPD patient with neither HUS nor HA). Representative profiles are shown in Fig. 1. Trf profiles from the three tested P-HUS and the P-HA patients showed multiple abnormal hypoacidic spots highly suggestive of hyposialylated glycoforms. The Trf profile appeared normal for the IPD patient with neither HUS nor HA.

Gal-3 plasma concentrations

The normal values of Gal-3 in plasma from 29 volunteer blood donors were 1.2 ± 0.6 ng/mL (Fig. 2). Plasma concentrations of Gal-3 were substantially higher in all IPD patients (Fig. 2 and Table 4) and reached 30.7 ± 8.2 ng/mL in the five P-HUS patients, 19.6 ± 10.3 ng/mL in the three P-HA patients, and 11.8 ± 4.7ng/mL in the two IPD patients with neither HUS nor HA.

DISCUSSION

Ten consecutive patients with IPD were enrolled in this study during a 3-year period. Meningitis was found in nine of these patients reflecting the particular recruitment of our laboratory. Five of these pneumococcal infections were complicated by full-blown HUS and three by pneumococcal-associated thrombocytopenia and HA (P-HA).

Several reports have described the occurrence of microangiopathic HA with minimal renal involvement in IPD patients.[28,29] In a retrospective study of 36 patients with IPD, Huang and coworkers[7] found 13 cases of P-HUS and nine cases of P-HA with microangiopathic changes on blood smear not associated with uremia. Similarly, in this study we report three P-HA cases that do not meet the strict criteria of HUS.

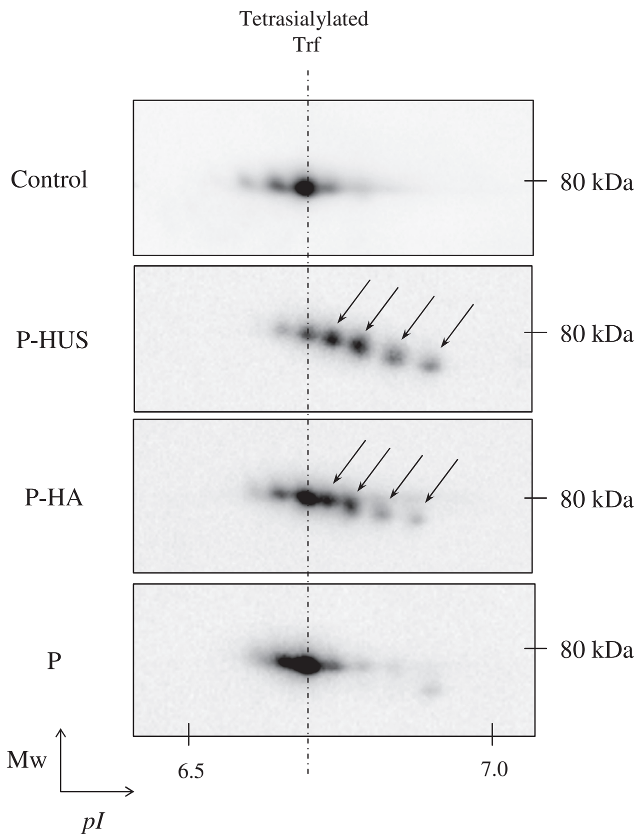


Fig. 1 Two-dimensional Western blot patterns of plasma transferrin. In the control, transferrin is mostly tetrasialylated and carries two typical biantenary N-glycan structures. In P-HUS (Patient 2) and P-HA (Patient 6) patient samples, multiple hypoacidic spots (arrows) strongly suggest hyposialylated glycoforms. In IPD patients with neither HUS nor HA (P, Patient 10), the Trf pattern is normal.

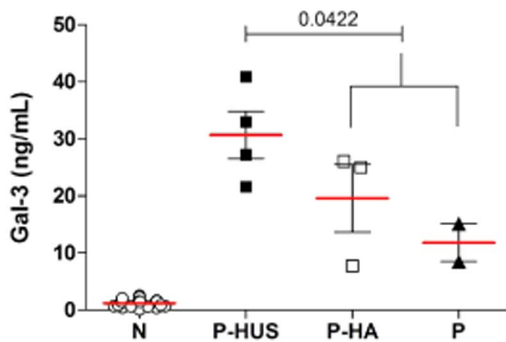


Fig. 2 Determination of Gal-3 plasma concentrations. Data are expressed as mean ± SEM in: volunteer blood donors (N), P-HUS, P-HA, and IPD patients with neither HUS nor HA (P).

Two patients (Patients 6 and 7) with peak creatinine concentrations of 88 and 60 μmol/L, respectively, showed no RBC microangiopathic anomalies on peripheral blood smear. In another case (Patient 8) RBC schizocytosis was present but creatinine values remained normal. The early recognition of P-HUS is imperative for adequate treatment but consistent case definition and diagnostic criteria are currently lacking. T-antigen testing is not always available; therefore, a positive DAT has been proposed as evidence of P-HUS.[2,30]

In this series, we found that DAT was positive in only three out of five P-HUS patients and in two out of three P-HA patients. This proportion is similar to the 61% of 207 cases reported in a recent review.[31]

Anti-T are complement-fixing[9] IgM isotype antibodies. These antibodies are considered to be present beyond 3 months of age and to reach adult levels by the age of 2 years.[11] Hence, anti-T may explain DAT positivity with anti-C3d in two patients (Patients 2 and 8). These two children with C3d+ DAT were 21 and 11 months old, respectively.

The weak IgG+ DAT positivity in three other patients (Patients 1, 3, and 6) is unlikely to be related to anti-T. We controlled several lots of anti-IgG reagents used in this study and did not find any positivity with in vitro neuraminidase-treated RBCs. Thus, IgG+ DAT positivity was not a consequence of contaminating anti-T present in rabbit sera used to produce antiglobulin reagents, as previously reported.[32]

More probably, IgG+ DAT positivity was secondary to intravenous (IV) antibiotic therapy. Indeed, most children received large doses of IV third-generation cephalosporins (cefotaxime or ceftriaxone), a treatment frequently associated with nonspecific adsorption of proteins on RBCs and positive DAT.[33]

By contrast with DAT, RBC T-antigen testing with both PNA and SBA lectins was 100% sensitive for the diagnosis of P-HUS as previously reported.[13] Furthermore, titrations with PNA showed a strong expression of T-antigen in all P-HUS and P-HA patients. The results indicate levels of desialylation of higher than 70%, since the reactivity of RBCs with PNA lectin first occurs when 20% to 25% sialic acid is released from RBC membrane and is maximum when 70% to 80% sialic acid is released.[34]

Most investigators used only PNA for T-activation diagnosis in P-HUS.[7,11,12] Although we analyzed only two uncomplicated IPD patients, our data suggest that the specificity of T-antigen testing for P-HUS diagnosis can be significantly improved by using a combination of lectins as described here or by quantifying T-antigen expression. The two IPD patients without any sign of P-HUS or P-HA exhibited a weak form of T-antigen expression called Th, which is defined by a positive agglutination test with PNA and no agglutination with SBA lectin.[5,8,35] Moreover, SBA is useful to differentiate T from Tk cryptantigen.[8,35] The Tk antigen is frequently exposed in IPD by the action of pneumococcal β-galactosidases.[5] In this report, Tk antigen was expressed in three of five P-HUS and three of three P-HA patients.

15372995, 2015, 6pt2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/trf.12981 by Arnaud Brunel - Assistance Publique Hopitaux De Paris Ap-Hp , Wiley Online Library on [20/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

To discriminate high levels of desialylation in P-HUS and P-HA patients, we analyzed sialic acid on RBCs with MAA lectin that recognizes sialic acid in the (α-2,3) linkage. We found significant differences in desialylation between patients and control RBCs; reciprocal agglutinin titers with MAA lectin were at least fourfold lower than controls in three of five P-HUS patients and in one of three P-HA patients (Patient 7).

The results obtained on RBCs from P-HUS patients and on control RBCs treated with various concentrations of neuraminidase suggest that differences in neuraminidase activity among P-HUS patients are as high as 100-fold. This observation is consistent with the findings of de Loos and colleagues[36] who measured neuraminidase activity during the acute phase in five P-HUS patients and found a wide range of values, varying from 0.2 to 32.3 nmol/hr/mL.

The analysis of Trf N-glycosylation profiles of five patients showed a good correlation with the results obtained for RBCs. Similar to the findings of de Loos and colleagues,[36] two-dimensional profiles of plasma Trf from patients with P-HUS (n = 3) or P-HA (n = 1) both showed multiple abnormal hypoacidic spots (cathodal shift) which are a strong indication of neuraminidase activity affecting α2,6-linked terminal sialic acids from N-glycoproteins (Fig. 1).

Overall, despite a wide range of clinical heterogeneity, P-HUS and P-HA patients were quite similar based on their RBC T-antigen expression and Trf N-glycosylation profiles. These results suggest that some additional, as yet unidentified, factors other than neuraminidase are involved in the development and the clinical presentation of P-HUS.

Relatively little is known on the mechanisms leading to P-HUS. Recent reports suggest that transient abnormalities of the alternative complement pathway are present in IPD and may contribute to the development of P-HUS.[14,15] Szilagyi and coworkers[14] found that the activity of the C3, C4, classical, and APC was transiently impaired in five P-HUS patients and they identified genetic mutations in complement genes in three of five patients. A good response to treatment with the complement inhibitor Eculizumab was reported in another P-HUS patient.[15] The possible mechanisms postulated include either low[14] or excessive[15] affinity of complement factor H (CFH) to desialylated endothelial cells, mutations of complement genes,[14] and neutralization of CFH by soluble pneumococcal proteins such as pneumococcal surface protein C.[6,15] CFH, the most important soluble regulator of the APC, binds to specific polyanionic structures on host cells in combination with the adjacent C3b-cell surface complex. This binding inhibits further deposition of C3b.[37] CFH contains at least two binding sites for heparin and polyanionic carbohydrates present on host cell surfaces, glycosaminoglycans, and other negatively charged molecules such as sialic acids.[37,38] Desialylation is a conceivable trigger for APC dysfunction because most human cells and tissues contain a high abundance of sialic acid–containing polysaccharides. However, CFH binding to endothelial cells involves predominantly glycosaminoglycans such as heparan sulfate[39] and sialic acids play a limited role.[38] For this reason, it is uncertain that neuraminidase activity may directly activate the APC on endothelial cells.

Given the activation of the APC by desialylated RBCs[16,17] and the reported role of Gal-3 in pneumococcal infections,[24] we hypothesized that adhesive interactions between desialylated blood cells and the endothelium trigger P-HUS by promoting localized complement activation and endothelial injury. This hypothesis prompted us to examine Gal-3 plasma concentrations in IPD patients.

We found that the plasma concentration of Gal-3 was substantially increased in all patients with P-HUS at the acute phase of the disease. In addition, Gal-3 concentrations were significantly higher in P-HUS patients than in other IPD patients, namely, P-HA patients and uncomplicated IPD patients (Fig. 2). However, Gal-3 is increased in most infectious and inflammatory diseases.[40] Further studies are needed to determine if Gal-3 is involved or not in the pathogenesis of P-HUS.

In conclusion, we found that T-antigen is consistently and strongly expressed on RBCs in a series of five P-HUS and three P-HA patients. This finding confirms the central role of neuraminidase activity in both conditions and the sensitivity of T-antigen testing for the early biologic diagnosis of P-HUS. Conversely, DAT was much less sensitive for P-HUS diagnosis.

PNA agglutination method is not specific for T-activation. Incomplete forms of T-activation (Th) do occur in uncomplicated IPD and Tk cryptantigen, exposed by pneumococcal β-galactosidase, is frequently present. Therefore, PNA agglutination positivity needs to be confirmed with SBA lectin to improve the specificity of T-activation for P-HUS diagnosis.

Further studies are required to elucidate the different pneumococcal or host-related factors associated with HUS in IPD patients in addition to pneumococcal neuraminidase activity. The high plasma concentrations of Gal-3 that we found in P-HUS patients suggest that Gal-3 may be one of these factors.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

ACKNOWLEDGMENTS

NBR was the principal investigator and takes primary responsibility for the the article; EG, LC, MJ, and PG recruited the patients, collected the data, and reviewed the manuscript; GB, AB, and TD

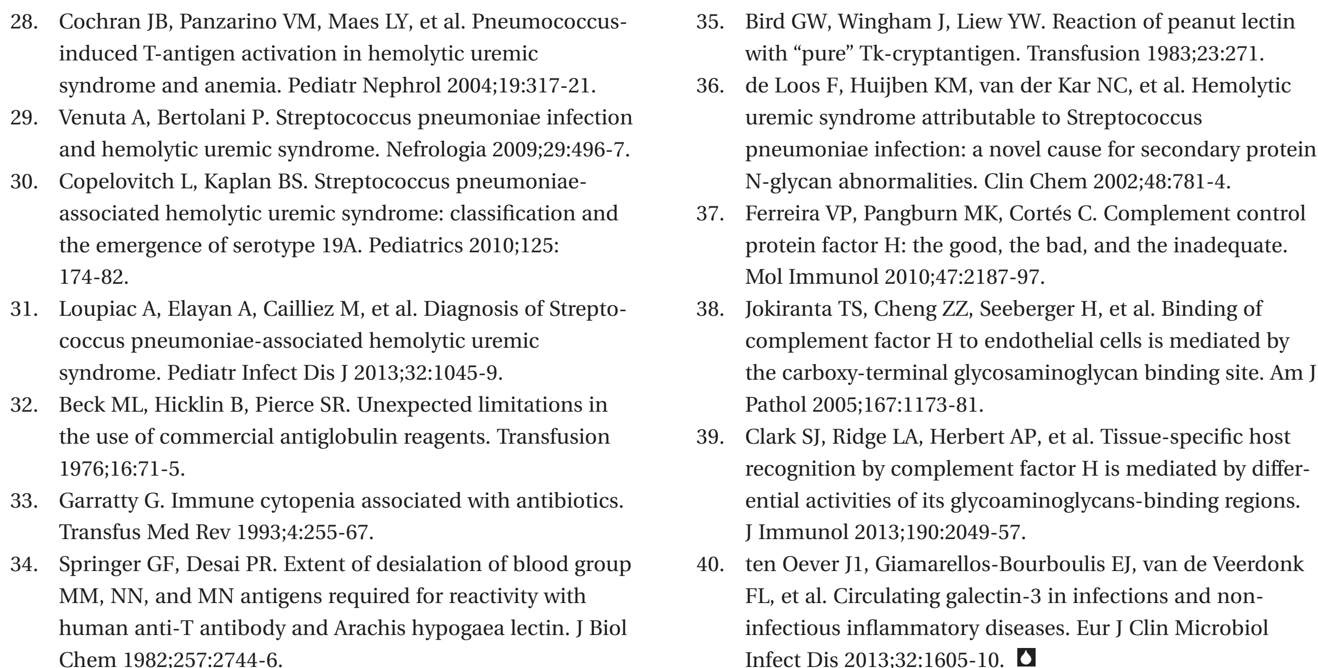
15372995, 2015, 6pt2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/trf.12981 by Arnaud Brunel - Assistance Publique Hopitaux De Paris Ap-Hp , Wiley Online Library on [20/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

performed the laboratory work for this study, analyzed the data, and reviewed the manuscript; PC provided statistical analysis; FNP and PB coordinated the research; NBR, PC, AB, and FNP wrote the manuscript.

REFERENCES

1. Copelovitch L, Kaplan BS. Streptococcus pneumoniae-associated hemolytic uremic syndrome. Pediatr Nephrol 2008;23:1951-6.
2. Spinale JM, Ruebner RL, Kaplan BS, et al. Update on Streptococcus pneumoniae associated hemolytic uremic syndrome. Curr Opin Pediatr 2013;25:203-8.
3. Coats MT, Murphy T, Paton JC, et al. Exposure of Thomsen–Friedenreich antigen in Streptococcus pneumoniae infection is dependent on pneumococcal neuraminidase A. Microb Pathog 2011;50:343-9.
4. Crookston KP, Reiner AP, Cooper LJ, et al. RBC T activation and hemolysis: implications for pediatric transfusion management. Transfusion 2000;40:801-12.
5. Daniels G. Polyagglutination and cryptantigens. In: Daniels G, editor. Human blood groups. 3rd ed. Oxford, UK: Wiley-Blackwell; 2013. p. 515-23.
6. Smith AJ, Johnston C, Inverarity D, et al. Investigating the role of pneumococcal neuraminidase A activity in isolates from pneumococcal haemolytic uraemic syndrome. J Med Microbiol 2013;62:1735-42.
7. Huang DT, Chi H, Lee HC, et al. T-antigen activation for prediction of pneumococcus-induced hemolytic uremic syndrome and hemolytic anemia. Pediatr Infect Dis J 2006; 25:608-10.
8. Bird GW, Wingham J. Vicia cretica: a powerful lectin for T- and Th- but not TK- or other polyagglutinable erythrocytes. J Clin Pathol 1981;34:69-70.
9. Burin des Roziers N, Bodivit G, Chadebech P, et al. Anti-T haemolysins: the effects of sialic acid removal and 2-aminoethylisothiouronium bromide treatment of erythrocytes on immune lysis. Vox Sang 2011;100: 401-8.
10. Klein PJ, Bulla M, Newman RA, et al. Thomsen-Friedenreich antigen in haemolytic-uraemic syndrome. Lancet 1977;2:1024-5.
11. Eder AF, Manno CS. Does red-cell T activation matter? Br J Haematol 2001;114:25-30.
12. Waters AM, Kerecuk L, Luk D, et al. Hemolytic uremic syndrome associated with invasive pneumococcal disease: the United Kingdom experience. J Pediatr 2007;151: 140-4.
13. Prestidge C, Wong W. Ten years of pneumococcal-associated haemolytic uraemic syndrome in New Zealand children. J Paediatr Child Health 2009;45:731-5.
14. Szilágyi A, Kiss N, Bereczki C, et al. The role of complement in Streptococcus pneumoniae-associated haemolytic uraemic syndrome. Nephrol Dial Transplant 2013;28:2237-45.
15. Gilbert RD, Nagra A, Haq MR. Does dysregulated complement activation contribute to haemolytic uraemic syndrome secondary to Streptococcus pneumoniae? Med Hypotheses 2013;81:400-3.
16. Fearon DT. Regulation by membrane sialic acid of BIH-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway. Proc Natl Acad Sci U S A 1978;75:1971-5.
17. Brown EJ, Joiner KA, Frank MM. Interaction of desialated guinea pig erythrocytes with the classical and alternative pathways of guinea pig complement in vivo and in vitro. J Clin Invest 1983;71:1710-9.
18. Sørensen AL, Rumjantseva V, Nayeb-Hashemi S, et al. Role of sialic acid for platelet life span: exposure of beta-galactose results in the rapid clearance of platelets from the circulation by asialoglycoprotein receptor-expressing liver macrophages and hepatocytes. Blood 2009;114: 1645-54.
19. Grewal PK, Aziz PV, Uchiyama S, et al. Inducing host protection in pneumococcal sepsis by preactivation of the Ashwell-Morell receptor. Proc Natl Acad Sci U S A 2013; 110:20218-23.
20. Bian CF, Zhang Y, Sun H, et al. Structural basis for distinct binding properties of the human galectins to Thomsen-Friedenreich antigen. PLoS ONE 2011;6: e25007.10.1371.
21. Di Lella S, Sundblad V, Cerliani JP, et al. When galectins recognize glycans: from biochemistry to physiology and back again. Biochemistry 2011;50:7842-57.
22. Glinsky VV, Glinsky GV, Rittenhouse-Olson K, et al. The role of Thomsen-Friedenreich antigen in adhesion of human breast and prostate cancer cells to the endothelium. Cancer Res 2001;61:4851-7.
23. Yu LG, Andrews N, Zhao QC, et al. Galectin-3 interaction with Thomsen-Friedenreich disaccharide on cancer-associated MUC1 causes increased cancer cell endothelial adhesion. J Biol Chem 2007;282:773-81.
24. Sato S, Ouellet N, Pelletier I, et al. Role of galectin-3 as an adhesion molecule for neutrophil extravasation during streptococcal pneumonia. J Immunol 2002;168: 1813-22.
25. Ahmad N, Gabius HJ, Andre S, et al. Galectin-3 precipitates as a pentamer with synthetic multivalent carbohydrates and forms heterogeneous cross-linked complexes. J Biol Chem 2004;279:10841-7.
26. Glinskii OV, Li F, Wilson LS, et al. Endothelial integrin α3β1 stabilizes carbohydrate-mediated tumor/endothelial cell adhesion and induces macromolecular signaling complex formation at the endothelial cell membrane. Oncotarget 2014;5:1382-9.
27. Bruneel A, Robert T, Lefeber D, et al. Two-dimensional gel electrophoresis of apolipoprotein C-III and other serum glycoproteins for the combined screening of human congenital disorders of O- and N-glycosylation. Prot Clin Appl 2007;1:321-4.

15372995, 2015, 6pt2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/trf.12981 by Arnaud Bruneel - Assistance Publique Hopitaux De Paris Ap-Hp , Wiley Online Library on [20/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License



28. Cochran JB, Panzarino VM, Maes LY, et al. Pneumococcus-induced T-antigen activation in hemolytic uremic syndrome and anemia. Pediatr Nephrol 2004;19:317-21.
29. Venuta A, Bertolani P. Streptococcus pneumoniae infection and hemolytic uremic syndrome. Nefrologia 2009;29:496-7.
30. Copelovitch L, Kaplan BS. Streptococcus pneumoniae-associated hemolytic uremic syndrome: classification and the emergence of serotype 19A. Pediatrics 2010;125: 174-82.
31. Loupiac A, Elayan A, Cailliez M, et al. Diagnosis of Streptococcus pneumoniae-associated hemolytic uremic syndrome. Pediatr Infect Dis J 2013;32:1045-9.
32. Beck ML, Hicklin B, Pierce SR. Unexpected limitations in the use of commercial antiglobulin reagents. Transfusion 1976;16:71-5.
33. Garratty G. Immune cytopenia associated with antibiotics. Transfus Med Rev 1993;4:255-67.
34. Springer GF, Desai PR. Extent of desialation of blood group MM, NN, and MN antigens required for reactivity with human anti-T antibody and Arachis hypogaea lectin. J Biol Chem 1982;257:2744-6.
35. Bird GW, Wingham J, Liew YW. Reaction of peanut lectin with “pure” Tk-cryptantigen. Transfusion 1983;23:271.
36. de Loos F, Huijben KM, van der Kar NC, et al. Hemolytic uremic syndrome attributable to Streptococcus pneumoniae infection: a novel cause for secondary protein N-glycan abnormalities. Clin Chem 2002;48:781-4.
37. Ferreira VP, Pangburn MK, Cortés C. Complement control protein factor H: the good, the bad, and the inadequate. Mol Immunol 2010;47:2187-97.
38. Jokiranta TS, Cheng ZZ, Seeberger H, et al. Binding of complement factor H to endothelial cells is mediated by the carboxy-terminal glycosaminoglycan binding site. Am J Pathol 2005;167:1173-81.
39. Clark SJ, Ridge LA, Herbert AP, et al. Tissue-specific host recognition by complement factor H is mediated by differential activities of its glycoaminoglycans-binding regions. J Immunol 2013;190:2049-57.
40. ten Oever J1, Giamarellos-Bourboulis EJ, van de Veerdonk FL, et al. Circulating galectin-3 in infections and non-infectious inflammatory diseases. Eur J Clin Microbiol Infect Dis 2013;32:1605-10.

15372995, 2015, 6pt2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/trf.12981 by Arnaud Brunel - Assistance Publique Hopitaux De Paris Ap-Hp , Wiley Online Library on [20/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License