Testing for CGD should be performed at any age whenever suspicious symptoms occur.

**References**


**Hemolytic Uremic Syndrome Attributable to Streptococcus pneumoniae Infection: A Novel Cause for Secondary Protein N-Glycan Abnormalities, Femke de Loos,1 Karin M.L.C. Huijben,1 Nicole C.A.J. van der Kar,2 Leo A.H. Monnens,2 Lambertus P.W.J. van den Heuvel,3 Johanna E.M. Groener,3 Ronald A. de Moor,3 and Ron A. Wevers1**

Hypoglycosylation of glycoproteins is characteristic for congenital disorders of glycosylation (CDG) and may consist of partial or completely missing glycans (1). The major first test for CDG is isoelectric focusing (IEF) of plasma transferrin. The IEF pattern shows a cathodal shift because of the hypoglycosylation of the protein (2). Primary defects in the N-glycan biosynthetic pathway are known for nine CDG subtypes: CDG Ia–f and CDG Ia–c (3–8). Transferrin hypoglycosylation can also be secondary to chronic alcohol abuse (9, 10), galactosemia (11), hereditary fructose intolerance (12), and severe liver...
In this report we describe the association of a transient abnormal transferrin N-glycosylation pattern in plasma and the clinical forms of hemolytic uremic syndrome (HUS) associated with a Streptococcus pneumoniae infection.

Five patients with HUS associated with a S. pneumoniae infection (age range, 9 months to 2 years) were studied. They fulfilled the criteria for HUS [hemolytic anemia with microangiopathic changes on peripheral blood smear (Burr cells), acute renal failure, and thrombocytopenia] and presented with pneumonia or meningitis. S. pneumoniae was isolated from body fluids of the five patients.

We also studied three patients with HUS attributable to verocytotoxin-producing Escherichia coli (VTEC) infection and one case of familial HUS of unknown etiology. von Willebrand factor cleaving protease deficiency and a defect in factor H were excluded in this patient.

The sialotransferrin fractions for the five S. pneumoniae-associated HUS cases and healthy controls are shown in Table 1 and Fig. 1. In healthy individuals (Fig. 1A, lane 1), the major transferrin form is the tetrasialo form. In all HUS cases associated with S. pneumoniae, the tetrasialotransferrin fraction was markedly decreased and the α-, mono-, and disialotransferrin fractions were increased (Fig. 1A, lane 7). The monosialotransferrin fraction showed the largest increase, followed by the asialo and disialo variants. The contribution of the trisialotransferrin isoform showed only a small increase in two cases. In the familial and VTEC-associated HUS patients, the transferrin isofocusing profile was normal (data not shown).

Other N-glycosylated plasma glycoproteins also have an abnormal isoform distribution in S. pneumoniae-associated HUS patients. Fig. 1B shows the electrophoretic pattern for N-glycosylated thyroxin-binding globulin (TBG) in a representative patient. In patient R (not shown), who also had the most abnormal transferrin IEF, no normal TBG isoforms could be detected and clearly abnormal TBG forms were present. These findings illus-

<table>
<thead>
<tr>
<th>Days after start of therapy</th>
<th>Neuraminidase activity, nmol·h⁻¹·mL⁻¹</th>
<th>Sialotransferrin fraction as determined by transferrin IEF, %</th>
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<tbody>
<tr>
<td>Controls (n = 30)</td>
<td></td>
<td></td>
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<td>CDG Ia (n = 12)</td>
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<tr>
<th>Patient</th>
<th>Days after start of therapy</th>
<th>Neuraminidase activity, nmol·h⁻¹·mL⁻¹</th>
<th>Sialotransferrin fraction as determined by transferrin IEF, %</th>
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<tbody>
<tr>
<td>H</td>
<td>Before</td>
<td>6.7</td>
<td>8.6  14.3  18.1  17.6  30.5  11.1  0.0</td>
</tr>
<tr>
<td>M</td>
<td>Before</td>
<td>2.6</td>
<td>6.5  14.4  21.2  15.0  29.4  12.0  1.6</td>
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<tr>
<td>L</td>
<td>Before</td>
<td>0.2</td>
<td>7.2  11.2  16.9  15.4  32.8  12.7  3.9</td>
</tr>
<tr>
<td>R</td>
<td>Before</td>
<td>32.3</td>
<td>15.3 37.0  36.2  8.2  3.3  0.0  0.0</td>
</tr>
<tr>
<td>R</td>
<td>30</td>
<td>0.1</td>
<td>2.7  1.8  5.0  10.1  52.6  20.9  6.9</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>13.9</td>
<td>12.6 14.5  22.7  24.4  21.0  4.8  4.8</td>
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<tr>
<td>B</td>
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<td>9.5  8.7  13.7  19.5  33.0  15.7  5.9</td>
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<tr>
<td>B</td>
<td>11</td>
<td>&lt;0.1</td>
<td>2.1  2.0  7.0  9.7  54.1  25.0  25.0</td>
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</table>

Table 1. Plasma neuraminidase activity (measured at pH 4.3) and plasma transferrin subfractions of HUS patients, CDG Ia patients, and healthy controls.

Fig. 1. Isofocusing of plasma transferrin (A) and TBG (B). (A), isofocusing polyacrylamide gels (pH 4–7; stained with Coomassie blue) of plasma transferrin. Lanes: 1, control; 2, CDG Ila; 3, CDG Ia; 4, fructose intolerance; 5, galactosemia; 6, alcohol abuse (double bands for each fraction can be explained by a frequent polymorphism in the transferrin protein); 7, HUS patient M. (B), isofocusing polyacrylamide gels (pH 4–6.5; silver stained) of plasma TBG. Lanes: 1, control; 2, HUS patient M.
trate that there is a more generalized glycoprotein abnormality in this disease. TBG isoform distribution was unremarkable in the familial and VTEC-associated HUS patients (data not shown).

The most striking alteration in the transferrin profile was observed in patient R, who had no more than 3.3% tetrasialotransferrin and very high percentages of di-, mono-, and asialotransferrin isoforms (Table 1). In this patient, *S. pneumoniae*-associated HUS was successfully treated with antibiotics. The transferrin IEF from the sample taken after 1 month of treatment showed a normal profile with tetrasialotransferrin as the major fraction and with normal isoform distribution (Table 1). In patient B, the transferrin profile gradually normalized within 11 days (Table 1). Similar data were obtained for TBG. Because the abnormal transferrin isoform distribution is secondary to the *S. pneumoniae* infection, it is not surprising that the glycoprotein abnormality was reversible after successful antibiotic treatment. These data show that this form of HUS is an as yet unrecognized secondary protein N-glycosylation abnormality.

Plasma neuraminidase activity in patients with HUS associated with a *S. pneumoniae* infection was increased in all cases (Table 1). In contrast, plasma samples of HUS patients who did not have a *S. pneumoniae*-associated infection, i.e., who had familial or VTEC-based HUS, showed almost undetectable neuraminidase activity. The neuraminidase activity determined in the plasma of patient R was the highest, corresponding to the very severe changes in the transferrin isoform distribution. The plasma sample from patient L showed the lowest neuraminidase activity among the five *S. pneumoniae*-associated HUS cases, whereas the transferrin profile of this patient was only slightly abnormal (Table 1). These findings suggest a relationship between the increase in neuraminidase activity and the extent of the alterations in the transferrin profile. The neuraminidase in plasma samples from *S. pneumoniae*-associated HUS patients was also active at neutral pH (40–50% of its activity at pH 4.3; data not shown). Therefore, it is likely that the neuraminidase may be active in the blood circulation of the patients as well.

Two N-glycosylated plasma proteins, transferrin and TBG, showed an abnormal isofocusing profile in *S. pneumoniae*-associated HUS patients. From our data it cannot be fully excluded that the *S. pneumoniae* infection interferes with protein N-glycan biosynthesis. However, it seems more likely that the glycoproteins are correctly synthesized and that they lose the sialic acid residues on the termini of their glycan antennas as a result of the action of circulating neuraminidase excreted by the *S. pneumoniae* (16). Neuraminidase produced and released by the *Streptococcus* is capable of removing sialic acid residues from glycoproteins present on the cell surface of erythrocytes, platelets, and glomerular endothelial cells, leading to hemolysis of erythrocytes, platelet agglutination, thrombocytopenia, and thrombotic microangiopathy (17). The effects of the high plasma neuraminidase will probably not be limited to the two glycoproteins investigated in this study. It seems likely that the high neuraminidase activity affects the glycosylation of many different glycoproteins. The glycans attached to the core of the protein play a role in the functional properties of the glycoprotein; thus, the undersialylation of the protein can limit the functionality of the protein. Perfusion studies on the effects of neuraminidase on kidneys showed progressive detachment of the endothelium and the epithelium from the glomerular basement membrane (18). Therefore, high plasma neuraminidase activity may play a pivotal role in the development of clinical signs in *S. pneumoniae*-associated HUS patients. More research needs to be performed to investigate the possible relationship between undersialylation of glycoproteins and clinical symptomatology of the patient. Our findings may help to unravel the pathophysiology of *S. pneumoniae*-associated HUS.

Changes in the plasma transferrin profile may result from various causes. CDG forms a group of inherited metabolic diseases with primary defects in N-glycan biosynthesis. In CDG type I, a- and disialotransferrin are increased. The asialotransferrin in CDG type I fully lacks its two potential glycans, whereas the asialotransferrin of a HUS patient lacks only the terminal sialic acid residues of its glycan(s). Transferrin isofocusing and other techniques that separate on the basis of isoelectric point are unable to discriminate between these two asialotransferrins, but the abnormal transferrin isofocusing pattern in *S. pneumoniae*-associated HUS, with increased a-, mono-, and disialotransferrin, is different from other known abnormal transferrin patterns, such as those found in CDG, alcohol abuse, or galactosemia (Fig. 1A). It therefore is possible to distinguish HUS from these clinical entities on the basis of transferrin IEF.

The typical pattern in CDG I patients shows increased amounts of a- and disialotransferrin and decreased amounts of tetrasialotransferrin, with normal amounts of mono- and trisialotransferrin (Fig. 1A, lane 3). Lane 2 in Fig. 1A shows the characteristic profile for a CDG IIa patient with highly increased disialotransferrin and almost absent tetrasialotransferrin. Also shown in Fig. 1A are the IEF transferrin profiles for alcohol abuse (lane 6, double bands attributable to a polymorphism in the protein part of transferrin), for galactosemia (lane 5), and for fructose intolerance (lane 4). These secondary causes for protein hypoglycosylation share characteristic abnormalities in the transferrin isofocusing profile with CDG type I. In this report we have used “hypoglycosylation” to describe the N-glycosylation defect in CDG cases, whereas “hyposialylation” seems more appropriate to describe the N-glycosylation abnormality in HUS patients.

Hypoglycosylation of transferrin is used as a diagnostic test for chronic alcohol abuse. Carbohydrate-deficient transferrin (CDT) is determined by electrophoretic or chromatographic procedures, e.g., IEF, capillary zone electrophoresis, HPLC, or anion-exchange chromatography on microcolumns; separated from the non-CDT, and subsequently detected. CDT analysis methods, such as the CDTect assay or the ChronAlcol.D assay, are expected to
give false-positive results in *S. pneumoniae* cases because only the amount of CDT (a- to di- or trisialotransferrin) and/or the CDT/transferrin ratio is determined (19). HUS associated with *S. pneumoniae* therefore has to be considered in the diagnosis of primary and secondary defects in the glycan part of N-glycoproteins.

The non-*S. pneumoniae*-associated HUS cases showed normal isoform distribution in both glycoproteins investigated in this study. This means that IEF of plasma transferrin distinguishes between *S. pneumoniae*-associated HUS and the two other HUS forms. This technique may therefore be complementary to the conventional bacteriologic techniques that are available for this purpose and may help to avoid the use of blood transfusions which would be life-threatening in cases of HUS associated with a *S. pneumoniae* infection.

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**References**


Can Glycohemoglobin Be Used to Assess Glycemic Control in Patients with Chronic Renal Failure? Randie R. Little,1,2* Alethea L. Tennill,1 Curt Rohlfing,1 Hsiao-Mei Wiedmeyer,1 Ramesh Khanna,3 Alok Agrawal,4 Richard Madson,5 and David E. Goldstein,2,3 (Departments of 1Child Health, 2Pathology and Anatomical Sciences, and 3Internal Medicine, University of Missouri School of Medicine, 1Hospital Dr., Columbia, MO 65212; 4Kidney & Hypertension Center, 1210 Hicks Blvd., Fairfield, OH 45014; 5Renal Physicians, Inc., 4700 Springboro Pike, Dayton, OH 45439; 6Department of Statistics/Biostatistics, 223 Math Sciences Bldg., University of Missouri, Columbia, MO 65211; *ad-dress correspondence to this author at: Department of Child Health, M767, University of Missouri School of Medicine, 1Hospital Dr., Columbia, MO 65212; fax 573-884-8823, e-mail LittleR@health.missouri.edu)

Many factors can affect interpretation of glycohemoglobin (GHB/HbA1c) measurements in patients with chronic renal failure (CRF). Several reports have suggested that erythrocyte survival is substantially lowered in most patients with CRF; this would be expected to lower GHB results (1–6). Several reports have also suggested that GHB methods, especially those based on charge separation (e.g., ion-exchange HPLC), may have interference by carbamyalted hemoglobin that would be expected to falsely increase GHB results (7–17). Many of these reports evaluated older assay methods; newer ion-exchange methods may show improved separation of the HbA1c fraction from other hemoglobin adducts (15, 17). Because renal failure is common in patients with diabetes and GHB is widely used as an index of mean blood glucose in these patients, we examined GHB results in patients with CRF by several different GHB assay methods. We also investigated the impact of shortened erythrocyte lifespan by comparing the GHB results obtained for nondiabetic patients with and without CRF.

Fifty-five patients with CRF (blood urea nitrogen >400 mg/L) were recruited for this study. Twenty-nine were not receiving dialysis and were seen at the University of Missouri Nephrology Clinic; the remaining 26 had end stage renal disease and were receiving hemodialysis at the Dialysis Clinic. Patients with renal failure included those with and without diabetes (*n* = 28 and 27, respectively). The GHB concentration (reported as percentage of HbA1c or equivalent) ranged from 4.4% to 11.2%. Informed consent was obtained from all participants according to