Table 1. Positivity rates for HSV1/2 RT-PCR and serologic testing (IgM and IgG) from May to July 2018 at MCL.

HSV test ordered	Number of tests performed	Positivity rates, %
RT-PCR alone	9999	HSV1, 15.7
		HSV2, 14.2
IgM alone	4584	2.3
IgM and IgG serologic panel	8100	IgM only, 0.6
		HSV1 IgG only, 48.8
		HSV2 IgG only, 21.6
		HSV1 IgG and HSV2 IgG, 13.4
IgM and RT-PCR ^a	171	IgM and HSV1 and/or HSV2 IgG, 1.5
		RT-PCR only, 49.7
		IgM only, 1.2
		RT-PCR and IgM, 4.7

^a Serum and swab samples collected within 7 days of each other.

overutilization and decrease laboratory resources (e.g., technologist time, kit cost) associated with performing these assays.

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S. Jung, statistical analysis.

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Beta-2 Transferrin Interference by a Neuraminidase Producing Microorganism

TO THE EDITOR:

Beta-2 transferrin is a desialylated transferrin isoform found almost exclusively in the cerebrospinal fluid (CSF). Electrophoresis and transferrin-specific immunofixation can be used to separate β -2 transferrin from the sialic acid-containing isoform found in serum. Detection of β -2 transferrin is a sensitive diagnostic method for CSF leakage (1). Beta-2 transferrin is distinct from the CSF marker sometimes known as beta-trace protein (or prostaglandin-D synthase).

Here we describe a case of altered transferrin electrophoretic migration, associated with infection by a neuraminidase-producing microorganism, an underrecognized cause of interference with this assay.

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Fluid was submitted for β -2 transferrin testing from a pediatric patient who had recently undergone craniofacial surgery. Postsurgical course was complicated by concern for infection and CSF leakage, and the specimen was collected during surgical reexploration. Agarose electrophoresis followed by immunofixation with antiserum to human transferrin was performed. The expected β -2 transferrin band was seen in the CSF positive control (Fig. 1, lanes 2 and 13), but not healthy human serum negative control (Fig. 1, lanes 1 and 14). In contrast, an atypical laddering pattern was present in the fluid specimen (Fig. 1, lanes 4 and 5). Transferrin polymorphisms can complicate interpretation of this assay, but the patient's serum did not demonstrate a transferrin polymorphism (Fig. 1, lane 3). The presence of several atypical transferrin bands complicating determination of CSF leakage was reported, and the test was nondiagnostic. Microbiologic cultures subsequently grew *Abiotrophia defectiva* and *Candida albicans*, which were treated with antimicrobial therapy.

Beta-2 transferrin represents the asialo form of this protein, which has a distinct electrophoretic migration compared to the major isoform in serum, containing 4 sialic acid groups. Neuraminidase (sialidase)-mediated reduction of the dominant serum isoform has been observed in the

Fig. 1. Transferrin immunofixation electrophoresis.

The dashed line indicates the cathodal edge of the beta-2 transferrin band in the control lanes. Transferrin laddering is present in the fluid specimen (lanes 4 and 5) and when healthy human serum is incubated with *Abiotrophia defectiva* (lanes 7 and 9).

setting of bacterial infection (1, 2). *Candida albicans* is reported to lack neuraminidase activity (3). However, *Abiotrophia defectiva*, previously named *Streptococcus defectivus*, does produce neuraminidase (4), suggesting that infection with this organism could have been responsible for the transferrin laddering we observed.

To determine if the presence of *Abiotrophia defectiva* is sufficient to cause step-wise transferrin desialylation and laddering, we

performed transferrin immunofixation on healthy human serum after incubation with this organism. Clinical *Abiotrophia defectiva* isolates were cultured on chocolate agar and suspended in tryptose phosphate broth to give an absorbance at 600 nm of 0.5. We incubated healthy human serum in tryptose phosphate broth alone (Fig. 1, lane 6) or with *Abiotrophia defectiva* suspensions (Fig. 1, lanes 7 and 9). We tested 2 different

clinical isolates of *Abiotrophia defectiva* and found that both were sufficient to produce a transferrin ladder pattern similar to that observed in the patient specimen. Transferrin bands were only detected by immunofixation when human serum was present in the incubation, thus indicating that the bands were not simply due to cross-reaction with bacterial proteins (Fig. 1, lanes 8 and 10).

Abiotrophia defectiva produces extracellular neuraminidase activity (4). We hypothesized that supernatant from bacterial cultures would also be sufficient to cause transferrin desialylation. We incubated the 2 *Abiotrophia defectiva* isolates in tryptose phosphate broth and prepared culture supernatants by centrifugation. Incubation of healthy human serum with culture supernatant was also sufficient to produce desialylated transferrin bands (Fig. 1, lanes 11 and 12). Together, these findings demonstrate that neuraminidase-producing organisms are sufficient to produce atypical transferrin bands that may interfere with interpretation of the β -2 transferrin assay.

In addition to *Abiotrophia defectiva*, a number of other microorganisms have been described to produce neuraminidase, which can serve as a virulence factor and promote infection or colonization (5). Both type A and B influenza viruses contain a

transmembrane neuraminidase in the viral envelope. By cleaving sialic acid residues in mucin, influenza neuraminidase facilitates viral movement to a target cell for infection. In addition, neuraminidase is critical for release of new viral particles from infected cells. *Streptococcus pneumoniae*, a common bacterial pathogen, also produces neuraminidase that promotes adherence to epithelial cells and growth on mucin. Gastrointestinal commensals such as *Ruminococcus gnavus* and pathogens including *Clostridium perfringens* also produce neuraminidase. These organisms utilize the enzyme to break down sialic acid in mucin to then use as a source of energy. Overall, the production of neuraminidase is not specific to *Abiotrophia defectiva*, and infections with these microorganisms could also cause a similar interference in this assay.

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