Congenital Syphilis: Detection of *Treponema pallidum* in Stillborns

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Paraffin-embedded tissue from all 17 autopsies performed following 56 stillbirths associated with maternal syphilis during a 3-year period (1987–1989) was reexamined to compare immunofluorescent antigen (IFA) testing with silver staining for the detection of *Treponema pallidum*. Congenital syphilis (CS) originally was diagnosed in 9 of the 17 cases of stillbirth, on the basis of positive silver stains (7 cases) or morphological findings alone (2). Upon review, silver staining revealed *T. pallidum* in 10 of 17 cases and IFA testing revealed the pathogen in 15 of 17 cases, enabling diagnosis of CS in 16 of 17 cases of stillbirth associated with a reactive maternal rapid plasma reagin (RPR) card test. Most stillbirths associated with a reactive maternal RPR test during this time period involved CS, and IFA testing for *T. pallidum* is superior to silver staining for the identification of treponemes.

The recent increase in maternal syphilis has led to an increase in congenital syphilis (CS) [1, 2]. CS is associated with significant mortality, and most deaths occur as stillbirths rather than among live-born infants [3, 4].

The Centers for Disease Control and Prevention (CDC) define a syphilitic stillbirth as a fetal death in which the mother had untreated or inadequately treated syphilis at delivery. The fetus must be the product of at least a 20-week gestation or weigh at least 500 g [2]. This surveillance definition is useful for epidemiological purposes but may not be accurate in individual cases, for which an autopsy provides a more definitive diagnosis.

An important aspect of the definitive diagnosis of CS by autopsy in a case of stillbirth is the silver stain, which has been the mainstay for detecting treponemes over the past 75 years [5]. Findings described in recent reports [6, 7] have continued to support well-documented [5] gross and microscopic features such as hepatosplenomegaly, perivascular fibrosis, and chorioamnionitis, as well as periendocarditis, in the diagnosis of CS following stillbirth. Placental and umbilical findings also continue to be important in the diagnosis of CS [8–13]. However, many stillborn fetuses with CS are macerated, and diagnostic morphological changes are often not present [14]. It is also unusual to find a macerated stillborn fetus teeming with spirochetes [7], and the performance and interpretation of silver stains remain arduous.

With the advent of newer staining techniques such as immunofluorescent antigen (IFA) testing [15], it has become easier to detect *Treponema pallidum* in genital lesions and various biopsy materials [16, 17], but the use of IFA testing in the diagnosis of CS has been limited [13, 18–21]. Our study was undertaken to compare IFA testing with silver staining in the diagnosis of CS in stillborns and to evaluate the specificity of the CDC surveillance definition for CS in stillborns.

Materials and Methods

From January 1987 through December 1989, there were 392 stillbirths at Kings County Hospital Center, a municipal hospital in Brooklyn, New York. In 17 (30%) of the 56 cases of stillbirth associated with a maternal reactive RPR (rapid plasma reagin) card test, confirmed by a reactive FTA-ABS (fluorescent treponemal antibody–absorbed) test, autopsies were performed. The original diagnosis of CS in these cases of stillbirth was made on the basis of morphological findings typical of CS, detection of treponemes with silver staining, or both. The original diagnosis of CS was not based on the CDC definition [2].

The autopsy reports as well as the medical records of the mothers of these stillborns were reviewed. Some maternal data from the other 39 cases of stillbirth associated with a reactive maternal RPR were reviewed for comparison. In addition, tissue sections from each of these cases were reviewed with use of hematoxylin and eosin, trichrome, and Dieterle (silver) staining as well as an IFA test for detection of treponemes (see below).

The organs most available for histological examination were the pancreas, liver, spleen, kidney, lung, and placenta. The IFA testing was performed on batches of tissues (a “run”) with a positive control (a slide from the FTA-ABS test kit) and a

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Guidelines for human experimentation at Kings County Hospital Center (Brooklyn, NY) were followed in the conduct of this clinical research.

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negative control (a patient known to be negative) in each run. Three negative controls—all randomly chosen stillborns, one from each of the 3 years, whose mothers’ RPR tests were nonreactive—were also included. The tissues were read blindly by the observer, who did not know the clinical status of the patient.

IFA Detection

Glass slides with formalin-fixed paraffin-embedded tissue sections were deparaffinized, covered with rabbit antiserum to *T. pallidum* (kindly provided by S. Lukehart, University of Washington, Seattle) diluted 1:100 with PBS (pH, 7.2), and incubated for 30 minutes under humid conditions. After being washed with gentle agitation in PBS for 5 minutes and air-dried, the slides were covered with the secondary antibody, rabbit IgG conjugated to fluorescein (Sigma, St. Louis; diluted 1:40 after reconstitution in PBS with 0.005% Evan’s blue as a counterstain), and incubated for 30 minutes under humid conditions.

The slides were washed in PBS for 5 minutes, washed in distilled water for 2 minutes, and air-dried. All slides were examined with an epifluorescence microscope (Nikon, Garden City, NY) with a 100× glycerol lens and standard coverslips with an antifading agent in buffered glycerol (0.1-M p-phenylenediamine/1-M Tris-HCl [pH, 8.0] in nine parts glycerol).

Statistics. Differences between the results of silver staining and IFA testing were calculated on the basis of confidence intervals of proportions and their differences, with use of the Confidence Interval Analysis computer program (version 1.0) [22]. An unpaired Student’s *t*-test was employed to compare RPR test titers.

Results

Association of Stillbirths with Reactive Maternal RPR and FTA-ABS Tests

During a 3-year period (1987–1989, inclusive), information on stillbirths at Kings County Hospital Center (Brooklyn, NY) was evaluated to determine the incidence of syphilitic stillbirths. In 1987, seven (5%) of a total of 145 stillbirths were associated with reactive maternal RPR and FTA-ABS tests. In 1988, 24 (17%) of 143 stillbirths and a total of 198 deliveries were associated with reactive maternal RPR and FTA-ABS tests. In 1989, 25 (24%) of 104 stillbirths and a total of 226 deliveries were associated with such reactive tests. In 1986, before the study, the proportion was only two of 123 stillbirths (2%).

Maternal Chart Review

The mean age of the RPR test–reactive mothers of autopsied stillborns was 26 years (range, 14–35 years). For these women, the mean RPR titer was 1:296; the median, 1:128; the mode, 1:128; the range, 1:1 to 1:2,048; and the geometric mean, 92. Fifteen of the 17 mothers had RPR titers of 1:32 or greater. For the group of 39 women who had reactive RPR and FTA-ABS tests and stillborns who were not autopsied, RPR test data were available for comparison: the mean titer was 1:107; median, 1:64; mode, 1:128; range, 1:1 to 1:512; and geometric mean, 34. There was no difference between the groups with respect to the geometric mean titer (*P > .05*).

The mean gestational age of the stillborns was 29.8 weeks; the median, 30 weeks; mode, 38 weeks; and range, 20–38 weeks. Prenatal care was documented for only one mother, who was also the only mother with a history of therapy during pregnancy, although four mothers reported a history of treatment before pregnancy. Adequate maternal therapy was documented for only two mothers, who were treated before pregnancy. One mother was noted to have the rash of secondary syphilis 2 weeks before delivery; the remaining 16 mothers had no information in their charts as to the stage of syphilis at delivery. The CDC definition of syphilitic stillbirths would have defined 15 of the 17 stillbirths as due to syphilis, excluding the two with adequate therapy.

A history of drug use was reported by 11 mothers, of whom 9 used crack/cocaine and 2 used heroin intravenously. Only one mother was known to be HIV-positive; for the rest there was no documentation of HIV testing.

Autopsy Report Review

Of the 17 stillbirths for which autopsy reports were available, the original diagnosis of CS was made in seven cases on the basis of detection of *T. pallidum* by silver staining of tissue sections. The diagnosis of CS made in two additional cases was based on the presence of classical morphological changes, despite a negative silver stain. Characteristic morphological findings and/or a positive silver stain was lacking in the remaining eight cases.

Silver Stain Review

Examination of the original silver stains confirmed the presence of spirochetes in each of the seven cases that were originally positive. In another three cases, on recutting of pathology material and very careful review, treponemes were found with silver staining. The tissue samples that yielded treponemes were from the pancreas, liver, and placenta. In each case only one type of tissue contained treponemes. One of these three cases had originally been diagnosed as CS on the basis of morphology alone; the other two cases had not been diagnosed as CS (table 1). All control specimens were negative.

IFA Detection Review

The IFA test was positive in all seven originally diagnosed cases and in an additional eight cases (table 1). Two of these
revealed the superiority of the IFA test in terms of the absolute RPR diagnosis of CS in 17 cases associated with a reactive maternal (P < .01). However, comparison of the two methods in the diagnosis of CS in 17 cases associated with a reactive maternal RPR did not show a difference between the two.

Discussion

A comparison of the silver stain and IFA detection methods revealed the superiority of the IFA test in terms of the absolute number of sections in which treponemes were detected and the ease of performance. The IFA test detected treponemes in an additional eight patients, and in six cases it was the only method that detected treponemes. The IFA test also revealed greater numbers of treponemes in the sections, and the results were much easier to read and interpret than those of the silver stain. These advantages may be due in part to the antifading compound.

To our knowledge, this is the first report of a large series of stillborns analyzed with use of IFA testing with minimal background immunofluorescent interference on full-thickness sections of formalin-fixed paraffin-embedded tissue. Past endeavors with IFA testing were either plagued with extreme background fluorescence or limited to the use of freshly ground or frozen tissue samples [16]. However, other investigators have recently used an IFA detection technique in the diagnosis of CS in selected cases, with favorable results [13].

In our series, the morphological characteristics of the organisms were more clearly seen with IFA testing, which made their identification as treponemes a less debated issue. In addition, the specificity of the antiserum increased the likelihood that these organisms were treponemes and not artifacts. The location of the treponemes within the tissue was also more recognizable than with Dieterle (silver) staining, as was evident in areas containing spirochetes within perivascular fibrosis.

The IFA detection technique was able to identify spirochetes in all but one of the stillborns who were ultimately diagnosed as having CS. In the one case of stillbirth in which spirochetes were visible only on silver staining, very few spirochetes were visualized. The reason for apparent failure of the IFA test in this case was probably that there were very few treponemes present. Therefore, any particular section may not have contained any organisms. This probably also explains why some of the repeated silver stains were positive, because different sections were cut and stained, which enabled the few treponemes present to be visualized. While it is not conclusive that the cause of death in each of these stillborns was CS, the fact that treponemes were identified in tissue proves that they were infected.

While all stillbirths should be followed by a postmortem evaluation because of possible implications for future births, only a minority of these stillborn babies whose mothers were

Table 1. Comparison of results of original and repeated silver staining and immunofluorescent antigen testing to detect treponemes in multiple tissues from 17 autopsied stillborns whose mothers had syphilis.

<table>
<thead>
<tr>
<th>Original SS result (no. of cases)</th>
<th>SS+</th>
<th>SS+</th>
<th>SS-</th>
<th>SS-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (7)</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative (10)</td>
<td>2</td>
<td>1*</td>
<td>6*</td>
<td>1</td>
</tr>
</tbody>
</table>

NOTE. IFA = immunofluorescent antigen; SS = silver staining; + = positive; - = negative.
* Two cases of stillbirth (one in each of these categories) were diagnosed as congenital syphilis on the basis of typical morphological findings, although silver stains were negative originally.

Table 2. Comparison of results of silver staining and immunofluorescent antigen testing of 116 tissue sections from 16 stillborns with congenital syphilis.

<table>
<thead>
<tr>
<th>IFA (no. of sections)</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver staining</td>
<td>41</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>22</td>
<td>51</td>
</tr>
</tbody>
</table>

NOTE. IFA = immunofluorescent antigen.
RPR-reactive were available for such evaluation, since many mothers had refused a request for autopsy. However, comparison of serological data from the two groups of mothers with reactive RPR tests and stillborns (autopsied vs. not autopsied) did not show a significant difference between the two groups. This suggests that the findings may be applicable to the group without autopsies.

In this series, evaluation of all stillbirths for evidence of *T. pallidum* was important even if the mother was treated, since treatment did not preclude CS by our definition (although the CDC definition of syphilitic stillbirths excludes stillbirths from the diagnosis of CS if the mother has received adequate therapy). Stains should be performed even when the material is macerated or autolyzed and/or morphological characteristics are absent, since the stain may still be positive and therefore diagnostic. Even if only the placenta is available, the identification of treponemes, usually in umbilical tissues, can be very helpful [13].

Stillbirths are more likely to be associated with early syphilis. Although only one mother had been found to have secondary syphilis, the high titers in most of these mothers suggested that they had early syphilis [23]. However, even a low titer and evidence of therapy did not preclude the diagnosis of CS in some of these stillbirths. In only one stillborn in this series was there no evidence of CS.

The association of crack/cocaine with maternal syphilis and CS in this series was consistent with previous observations [2]. Our study was conducted during the last epidemic of syphilis in New York. The numbers and percentages of stillbirths associated with reactive maternal RPR and FTA-ABS tests were very high during 1988–1990, and comparison with 1986 data demonstrates this well. High rates of stillbirths associated with maternal syphilis are usually seen in places such as Africa [24], where up to 39% of stillbirths in one study [25] were associated with a reactive maternal RPR test. One American study [26] performed during a similar time period revealed rates of CS among stillbirths ranging from 5.4% to 18%.

While it is possible that the surveillance definition may lack specificity for individual patients, the finding of treponemes in 16 of 17 stillbirths associated with a reactive maternal RPR test confirmed that the CDC surveillance definition of syphilitic stillbirths was specific for individual stillbirths in this study. However, this study was conducted during the most recent syphilis epidemic, when a reactive maternal serology was often reflective of active infection. The CDC surveillance definition may not be as accurate in nonepidemic situations. All stillbirths associated with reactive maternal RPR tests should be evaluated for CS with immunofluorescent and silver stains.

References