Pathogenesis and Diagnosis of Human Meningococcal Disease Using Immunohistochemical and PCR Assays

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**Abstract**

*Neisseria meningitidis* remains the leading cause of fatal sepsis. Cultures may not be available in fulminant fatal cases. An immunohistochemical assay for *N* meningitidis was applied to formalin-fixed samples from 14 patients with meningococcal disease. Histopathologic findings in 12 fatal cases included interstitial pneumonitis, hemorrhagic adrenal glands, myocarditis, meningoitis, and thrombi in the glomeruli and choroid plexus. Meningeal inflammation was observed in 6 patients. Skin biopsies of 2 surviving patients showed leukocytoclastic vasculitis and cellulitis. By using immunohistochemical analysis, meningococci and granular meningococcal antigens were observed inside monocytes, neutrophils, and endothelial cells or extracellularly. By using real-time polymerase chain reaction (PCR) on formalin-fixed tissue samples, meningococcal serogroup determination was possible in 11 of 14 cases (8 serogroup C, 2 Y, and 1 B). Diagnosis and serogrouping of *N* meningitidis can be performed using immunohistochemical analysis and PCR on formalin-fixed tissue samples. Immunohistochemical analysis determined the distribution of meningococci and meningococcal antigens in tissue samples, allowing better insights into *N* meningitidis pathogenesis.

*Neisseria meningitidis* remains the leading cause worldwide of fatal sepsis and sporadic cases of meningitis.1,2 The severity of symptoms and rapid clinical deterioration in the conditions of people with meningococcal sepsis require immediate empiric therapy, which initially is given on the basis of clinical suspicion and epidemiologic information.3-7 A definitive diagnosis of *N* meningitidis in index cases is important so that appropriate prophylaxis can be given to members of their households and other contacts.2 In addition, serogroup determination is important for vaccination and other public health interventions. The classic laboratory diagnosis has relied on culture of *N* meningitidis from sterile sites (eg, blood, cerebrospinal fluid [CSF]). However, the sensitivity of culture is low when performed after initiation of antimicrobial treatment, and cultures require 12 to 24 hours for growth; thus, alternative diagnostic methods, such as antigen detection with latex agglutination assays and polymerase chain reaction (PCR) have been developed.8-12 Various PCR approaches have been used on CSF, blood, and serum samples to detect *N* meningitidis in culture-negative cases.10-12

Meningococcal disease can have a nonspecific prodrome followed by the acute onset of fever, rash, and flu-like or gastrointestinal symptoms.3,6 On occasion, the first suspicion of meningococcal disease is when the postmortem examination reveals hemorrhagic adrenal glands (Waterhouse-Friderichsen syndrome [WFS]) or meningitis.13 Because the disease in fatal cases often progresses rapidly, specimens for culturing may not have been obtained before death, and formalin-fixed tissue specimens may be the only specimens available for analysis. Detection of bacteria in formalin-fixed tissue specimens traditionally has been done using Gram or silver impregnation stains, which are neither sensitive nor...
specific. Thus, diagnostic methods that permit specific detection of *N meningitidis* in postmortem formalin-fixed tissue samples, such as immunohistochemical analysis and PCR, can be of great value.

Immunohistochemical assays permit the detection of bacteria and their antigens while retaining tissue morphologic features. This permits the study of pathogenic mechanisms in human tissue samples, the only natural reservoir for *N meningitidis*, and can help validate observations that have been made in vitro. We describe the successful application of *N meningitidis* immunohistochemical analysis and serogroup-specific, real-time PCR assays using formalin-fixed tissue samples and show how these techniques can be used to better understand the pathogenesis of meningococcaldisease.

### Materials and Methods

Formalin-fixed, paraffin-embedded tissue specimens from 14 patients with meningococcal disease were evaluated at the Centers for Disease Control and Prevention (CDC), Atlanta, GA. The type of tissue received from each case is listed in Table 1. H&E and immunohistochemical staining were done on all tissue samples. Gram and Steiner stains were done on the paraffin blocks that showed the most abundant immunohistochemical staining.

### Immunohistochemical Analysis

Immunohistochemical assays were performed as previously described for other infectious agents. Tissue sections, 3 µm thick, were placed on Colormark Plus slides (Erie Scientific, Portsmouth, NH), deparaffinized, and rehydrated through graded alcohols and distilled water. Tissue sections were washed in pH 7.5 buffer containing tris(hydroxymethyl)aminomethane (Tris), saline, and polyborate (Tween) 20 (TST) and placed in an autostainer (DAKO, Carpinteria, CA). Digestion was performed by soaking slides in a 0.1-mg/mL concentration of Proteinase K (Roche, Indianapolis, IN) in 0.6 TST/0.1% calcium chloride (buffer) for 15 minutes at room temperature, followed by a blocking step with 20% normal sheep serum in TST. Tissue sections were incubated for 60 minutes with a polyclonal horse antibody prepared against *N meningitidis* serogroup C capsular polysaccharide (I.M. Feavers, National Institute for Biological Standard and Control, Potters Bar, England). Optimal dilutions of the antibodies had been determined by previous experiments on positive control tissue samples (the horse antibody was used at 1:500 and the murine antibody at 1:1,000). After being incubated with the primary antibody, slides were washed and a goat antihorse or a swine antimouse biotinylated link antibody, alkaline phosphatase–labeled streptavidin, and naphthol fast red chromogenic substrate (LSAB2 universal alkaline phosphatase system, DAKO) were applied sequentially. Sections then were counterstained in Mayer hematoxylin (Fisher Scientific, Pittsburgh, PA).

The polyclonal horse antibody against *N meningitidis* serogroup Y was used to screen all tissue samples because it cross-reacted against formalin-fixed, paraffin-embedded cultures of *N meningitidis* serogroups A, B, C, Y, and W135, *Neisseria lactamica*, *Neisseria cinerea*, *Neisseria subflava*, and 2 strains of *Neisseria gonorrhoeae*. This polyclonal antibody did not show cross-reactivity against formalin-fixed, paraffin-embedded cultures of group B streptococci, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Listeria monocytogenes* or against tissue sections from patients with confirmed cases of *Streptococcus pneumoniae* or *Mycobacterium tuberculosis* meningitis, toxic shock syndrome due to group A streptococci, or spotted fever group rickettsia. The monoclonal antibody against *N meningitidis* serogroup C reacted against formalin-fixed, paraffin-embedded cultures of *N meningitidis* serogroup C and did not cross-react with formalin-fixed, paraffin-embedded cultures of *N meningitidis* serogroups A, B, Y, and W135.

Negative controls for tissues screened with the polyclonal anti-*N meningitidis* antibody consisted of sequential, case tissue sections incubated with normal horse serum as the primary antibody. Negative controls for sections tested with the serogroup C antibody included incubation with mouse ascites fluid.

Interpretation of immunohistochemical results included evaluation of the following: (1) the location of the positive reaction, (2) the staining pattern (well-defined meningococci vs granular staining), and (3) the amount of staining (absent, rare, or abundant).

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tissue Sample</th>
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<tbody>
<tr>
<td>1</td>
<td>Brain, lung, heart, liver, adrenal</td>
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<td>2</td>
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<td>5</td>
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<tr>
<td>14</td>
<td>Brain, lung, heart, adrenal</td>
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Electron Microscopic Studies

Transmission electron microscopy was performed on formalin-fixed lung tissue samples from 2 patients and on choroid plexus samples from 2 other patients. The tissue fragments were placed for 1 hour in osmium tetroxide, dehydrated in graded alcohol, and infiltrated with propylene oxide. The tissue fragments then were embedded in Maraglas plastic (Electron Microscopy Sciences, Washington, PA) and polymerized at 60°C overnight. Sections were cut on an Ultratcut UCT microtome (Leica Reichert, Vienna, Austria) using a diamond knife, picked up on copper mesh grids, and poststained with uranyl acetate and lead citrate. The sections were examined with a transmission electron microscope (Philips Electronic Instruments, Mahwah, NJ) at 40 kV.

Polymerase Chain Reaction

DNA extraction of formalin-fixed tissue samples from 14 patients was performed with the QIAmp DNA mini kit (Qiagen, Valencia, CA). The DNA was eluted in 200 μL of a 10-mmol/L concentration of Tris (pH 7.5). Previously described real-time PCR assays were used for detection of ctra for N meningitidis and for the specific capsule genes of each of the serogroups (A, B, C, Y, W135, and X). Table 2 gives the nucleotide sequence for these genes.

Results

Epidemiologic and Clinical Data

Of the 14 patients, 3 were associated with a meningococcal outbreak in Taiwan, and 11 were from the United States (9 sporadic cases and 2 outbreak-associated cases). Table 3 presents pertinent clinical and laboratory information for the patients studied. Of the 14 patients, 12 (86%) were 21 years old or younger (median age, 12 years; range, 1-52 years), and only 4 (29%) were male. Except for 2 patients who survived, 9 patients died within 24 hours of admission, 2 died before arriving at a medical facility, and 1 died 4 days after hospital admission. One patient had systemic lupus erythematosus and was taking steroids; all other patients were previously healthy.

The availability of clinical information varied by patient; however, fever was documented in 13 (100%) of 13, skin rash in 10 (71%) of 14, gastrointestinal signs (nausea, vomiting, and/or diarrhea) in 8 (67%) of 12, and/or respiratory symptoms (influenza-like symptoms, sore throat, and/or cyanosis) in 7 (58%) of 12. Clinical meningeal signs, such as stiff neck, headache, photophobia, or seizures, were documented only in 4 (44%) of 9 patients. Meningococcal disease was suspected clinically in 11 patients; however, other clinical diagnoses were made in 3 patients (toxic shock syndrome, cellulitis, and Chagas myocarditis). The 2 patients who survived and one of the patients who died received antimicrobial treatment for more than 4 days. Data about antimicrobial treatment for the remaining patients was difficult to determine because they were never admitted or were treated for only a few hours in emergency departments.

Histopathologic Findings

Interstitial pneumonitis was found in 11 (92%) of 12 patients and consisted of mononuclear inflammation in the alveolar septa Image 1A, with little inflammation, edema, or hemorrhage inside the alveoli. Six of the patients with pneumonia had respiratory symptoms. Adrenal hemorrhage and necrosis, as a sign of WFS, occurred in 8 (80%) of 10 patients, 5 of whom had a rash. The amount of hemorrhage and necrosis in the adrenal glands varied from total effacement of the adrenal tissue Image 2A to small hemorrhages. Abnormal histopathologic features in the heart were found in 8 (80%) of 10 patient samples; in 7 of the samples, they consisted of small foci of neutrophilic inflammation accompanied by myocyte necrosis and edema (myocarditis) Image 3A, and in 1 case an endocardial vegetation with underlying myocardial inflammation was noted. Neutrophilic inflammatory infiltrate in the meninges was present in 6 (50%) of 12 cases; 3 had clinical signs of meningeal inflammation. Glomerular fibrin thrombi were observed in 4 (67%) of 6 cases. Skin samples...
from both surviving patients showed leukocytoclastic vasculitis \textbf{Image 4A}; in 1, the vasculitis was accompanied by marked mixed inflammatory infiltrate in the dermis and subcutis. A bone marrow sample from 1 patient showed focal areas of necrosis, hemophagocytosis, and left shift of the WBCs. In the only ovary studied, endothelial cells appeared to have sloughed off into the vascular lumen \textbf{Image 5A}.

Gram staining revealed clumps of definitive gram-negative cocci in samples from 5 patients. These patients had large bacterial clumps in blood vessels of the heart and central nervous system, and in 4 of the cases, the bacteria could be seen with H&E staining. Seven case samples had irregular structures suggestive of gram-negative bacteria inside circulating monocytes; however, definitive confirmation of the presence of cocci could not be made by the use of Gram stain in these cases. All cases showed intracellular rounded material inside mononuclear cells when Steiner silver stain was used; however, definitive identification of meningococci in all cases was difficult because silver staining structures, such as carbon particles, lipofuscin, or neurosecretory granules, precluded diagnosis.

\begin{table}[h]
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\small
\caption{Clinical Signs and Symptoms, Major Pathologic Findings, and Laboratory Tests Confirming Diagnosis of \textit{Neisseria meningitidis} in 14 Patients}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Case No./ Sex/Age (y) & Clinical Signs and Symptoms\textsuperscript{*} & Major Pathologic Findings & Positive for \textit{N meningitidis}\textsuperscript{†} & PCR Results/ Non–Formalin-Fixed Specimens/Test/Results & Outcome \\
\hline
1/F/19 & Fever, rash, gastrointestinal & Pneumonitis, myocarditis, WFS & Brain (blood vessels), lungs, heart, liver, adrenals & Serogroup C/lung, adrenal & Ear drainage/culture/ \textit{serogroup C} & Died \\
2/F/52 & Fever, rash, gastrointestinal, respiratory & Pneumonitis, WFS & Brain (blood vessels), lungs, liver & Serogroup C/lung & Blood/culture/ \textit{serogroup C} & Died \\
3/M/18 & Fever, gastrointestinal, respiratory & Pneumonitis, WFS, glomerular thrombi & Brain (blood vessels), lungs, heart, liver, spleen, adrenal, kidney & Serogroup C/lung, spleen & CSF/latex agglutination/ positive & Died \\
4/F/20 & Fever, rash, gastrointestinal & Myocarditis, WFS, glomerular thrombi & Brain (blood vessels), lungs, heart, liver, spleen, adrenal, kidney & Ctr\textsuperscript{‡}/lung, heart & Blood/culture/ \textit{serogroup Y} & \\
5/F/4 & Fever, rash, respiratory & Pneumonitis, myocarditis & Brain (blood vessels), lungs, heart, liver, kidney, intestine & Serogroup Y/heart & None & Died \\
6/M/2 & Rash & Pneumonitis, myocarditis, WFS & Brain (blood vessels), lungs, heart, liver, spleen, adrenal, kidney & Serogroup C/heart & Serum/PCR/ \textit{serogroup C} & Died \\
7/F/1 & Fever, rash, respiratory & Cellulitis & Skin & Serogroup C/skin & Skin lesions/culture/ \textit{serogroup C}; CSF/culture/negative & Survived \\
8/M/12 & Fever, rash, meningeval & Leukocytoclastic vasculitis & Skin & Serogroup B/skin & CSF/culture/negative & Survived \\
9/M/21 & Fever, rash & Meningitis, pneumonitis, myocarditis, WFS, glomerular thrombi & Brain (meninges and blood vessels), lungs, heart, liver, spleen, adrenal, kidney & Serogroup Y/heart, lung, brain & Blood/culture/ \textit{serogroup Y} & Died \\
10/F/7 & Fever, meningeval & Meningitis, pneumonitis & Meninges, spleen & Serogroup C/heart & CSF/culture/ \textit{serogroup C}; CSF/latex agglutination/ positive & Died \\
11/F/12 & Fever, gastrointestinal, respiratory, meningeval & Meningitis, pneumonitis, WFS & Meninges, lung, adrenal & Ctr\textsuperscript{‡}/lung, brain & CSF/latex agglutination/ Died & \\
12/F/21 & Fever, rash, gastrointestinal & Meningitis, pneumonitis, myocarditis & Brain (meninges and blood vessels), lungs, heart, bone marrow, ovary & Serogroup C/heart & Serum/ \textit{culture}/ \textit{serogroup Y} & Died \\
13/F/51 & Fever, rash, gastrointestinal, intestinal & Meningitis, pneumonitis, endocarditis, glomerular thrombi & Lung, heart, liver, spleen & Serogroup C/heart & CSF/culture/negative & Died \\
14/F/8 & Fever, gastrointestinal, respiratory, meningeval & Meningitis, pneumonitis, myocarditis, WFS & Meninges & Ctr\textsuperscript{‡}/lung, adrenal & CSF/culture and latex agglutination/negative; CSF/PCR/ \textit{serogroup B} & Died \\
\hline
\end{tabular}
\textsuperscript{*}Gastrointestinal signs included nausea, vomiting, diarrhea, and abdominal pain; respiratory signs included influenza-like symptoms, sore throat, and cyanosis.
\textsuperscript{†}Immunohistochemical staining using the anti-\textit{N meningitidis} serogroup Y antibody.
\textsuperscript{‡}\textit{ctr}A gene positive but no serogroup identified.
\end{table}

CSF, cerebrospinal fluid; PCR, polymerase chain reaction; WFS, Waterhouse-Friderichsen syndrome.
Immunohistochemical and Electron Microscopic Findings

Immunohistochemical analysis for *N meningitidis* showed intracellular and extracellular meningococci singly, in pairs, and in clumps in all 14 cases. Most of the cells that contained *N meningitidis* corresponded to circulating monocytes [Image 1B](#) (Image 2A, inset); polymorphonuclear leukocytes [Image 3B](#) and endothelial cells [Image 6A](#) also occasionally contained meningococci. Smaller fragments of granular bacterial antigen staining inside inflammatory [Image 2B](#) and [Image 4B](#) (Images 1B and 3B) and endothelial cells [Image 5B](#) and [Image 6B](#) also were demonstrated. Table 3 shows the distribution of meningococci in tissues shown by immunohistochemical analysis for each case. Large numbers of bacteria and amounts of antigen were observed in liver (8/8 [100%]), spleen (6/6 [100%]), and kidneys (5/6 [83%]).

In addition to meningococci staining in the renal blood vessels, immunohistochemical staining was seen in the glomerular thrombi. Meningococci and meningococcal antigens were observed frequently in the lungs (10/12 [83%]) and
heart (8/10 [80%]), but the amount of immunohistochemical staining was not always as extensive as in liver, spleen, and kidney. In the heart, immunohistochemical staining was observed in the inflammatory foci (Image 3B). Immunohistochemical bacterial staining in the lung correlated with pneumonitis in 9 cases and with respiratory signs (influenza-like symptoms, sore throat, and/or cyanosis) in 5 cases. In 6 (60%) of 10 cases, hemorrhagic adrenal cortices and meningococcal antigens (Image 2B) were observed frequently, while meningococci were not as abundant. Samples from the 2 surviving patients showed bacteria and antigens in blood vessels and capillaries and inside inflammatory cells present in the skin (Image 4B) and subcutaneous tissues.

Electron microscopic examination of the lungs of 2 patients (19-year-old woman and 2-year-old boy) demonstrated diplococci (Image 1C) and pleomorphic bacteria (Image 1D).
inside mononuclear cells in blood vessels. In some areas, the meningococci appear to have vesicle formations stemming from their outer membrane (meningococcal blebs) (Image 1E).

By using immunohistochemical analysis, we were able to observe meningococci and meningococcal antigens in the meninges in 3 cases, in the meninges and brain blood vessels (Image 6B) in 2 cases, and in brain blood vessels only in 6 cases. Five patients with meningococci and meningococcal antigens in the meninges had meningeal inflammation, except for case 13, who had histopathologic evidence of meningitis with negative meningococcal immunohistochemical staining. Case 13 had received antimicrobials for 4 days before death. Of the 4 patients with clinical signs of meningeal involvement, 3 patients died and had histologic evidence of meningitis with bacterial staining in the meninges; the fourth patient survived.
Choroid plexus samples were available for 2 patients (1 with meningitis) and showed abundant meningococci and meningococcal antigens in blood vessels; meningococci and meningococcal antigens were observed focally in the interstitium and the choroid plexus epithelium, particularly in areas where thrombosis had occurred. Electron photomicrographs of the choroid plexus in these 2 patients (4- and 21-year-old females) showed meningococci in the subarachnoid space, choroid plexus epithelium, interstitium, and blood vessels.

**PCR Findings**

Results from real-time PCR assays using nucleic acids extracted from formalin-fixed tissue samples are given in Table 3. Real-time PCR using primers and probes targeting the capsule transport gene, *ctrA*, of *N meningitidis* confirmed...
the presence of \textit{N meningitidis} in all cases. Serogroup-specific, real-time PCR assays were able to determine the \textit{N meningitidis} serogroup in 11 cases: 8 were serogroup C, 2 were serogroup Y, and 1 was serogroup B. Of the 3 cases in which the serogroup could not be determined using DNA extracted from formalin-fixed tissue samples, 2 had serogrouping done on cultures or CSF. In 1 case, the only material available for real-time PCR was skin biopsy tissue sections mounted on slides. Of the 8 cases of \textit{N meningitidis} serogroup C, 7 were confirmed using the serogroup C–specific immunohistochemical analysis. Other tests that confirmed \textit{N meningitidis} are given in Table 3. Of interest are 2 cultures from nonsterile sites (ear drainage and skin) that grew \textit{N meningitidis}, but the isolates were not considered significant because of the specimen sources.

**Discussion**

In this study, we demonstrated the presence of \textit{N meningitidis} in formalin-fixed tissue samples from patients with meningococcal disease by using a novel immunohistochemical assay and real-time PCR. The \textit{N meningitidis} serogroup was identified by using serogroup-specific, real-time PCR; immunohistochemical analysis permitted detection of \textit{N meningitidis} while preserving tissue morphologic features and permitted study of the pathology of meningococcal disease in human tissues. Routine methods for the detection of bacteria in formalin-fixed tissue samples, such as Gram and silver impregnation stains, can be difficult to interpret and are nonspecific; thus, formalin-fixed tissue samples have been considered unsuitable for performing specific detection of \textit{N meningitidis} or for determining the serogroup. The importance of being able to use formalin-fixed specimens for specific diagnosis is highlighted by the information obtained with the immunohistochemical assay performed on the skin biopsy specimens of the 2 surviving patients in our study. In one of these patients, empiric antimicrobial treatment likely prevented growth of \textit{N meningitidis}, and the specific diagnosis of meningococcemia was made by using the meningococcal immunohistochemical assay. In the second surviving case, the significance of the \textit{N meningitidis}–positive skin culture was not appreciated until the immunohistochemical assay demonstrated bacteria inside inflammatory cells in blood vessels, indicating a systemic process.

\textit{N meningitidis} colonizes the naso-oropharyngeal mucosa in 5% to 10% of persons in the United States, but invasive disease is rare (~1 case per 100,000 persons per year).2,3,17 For \textit{N meningitidis} to cause invasive disease, meningococci need to attach and invade the respiratory epithelium, enter the circulation, and multiply by evading bactericidal activity.1,3,18,19 By using immunohistochemical analysis and electron microscopy, we demonstrated that meningococci were inside circulating monocytes in fatal and surviving cases. These techniques also permitted identification of granular meningococcal antigens and bacterial membrane vesiculation or blebs inside these monocytes. In vitro studies have shown that \textit{N meningitidis} membrane fragments and outer membrane blebs containing endotoxin induce the production of proinflammatory cytokines (eg, tumor necrosis factor \(\alpha\), interleukins 1 and 6, interferon \(\gamma\)) by monocytes and neutrophils.1,20,22 In vivo, these cytokines have an important role, inducing an inflammatory response and causing shock and disseminated intravascular coagulation.

Internalization of meningococci in human monocytes, neutrophils, and epithelial and endothelial cells has been studied in vitro. This is a complex process involving interaction of meningococci with human cell membrane components that results in close adherence and internalization of the meningococci, often in phagocytic vacuoles.18,23,24 As meningococci are internalized, there is production, expression, and release of tissue factor and tissue plasminogen activator, which have both proinflammatory and procoagulant activities.3,25 This is supported by finding meningococci and meningococcal antigens within inflammatory foci in the heart and meninges and in thrombi present in the glomeruli and choroid plexus. In addition, the detachment of the endothelial cells from vessel walls could be induced by the internalization of meningococci in a manner similar to the detachment that occurs in rickettsial infections.26 Thus, by applying immunohistochemical analysis to human tissues, we demonstrated that meningococci and meningococcal antigens circulate inside monocytes, neutrophils, and endothelial cells, seeding and causing pathology in a variety of tissues.

In the fatal cases in the present study, interstitial pneumonitis, myocarditis, or WFS (shock associated with rash and adrenal insufficiency) were noted in more than 90% of the patients (11/12), while meningitis was present in only 50% (6/12). \textit{N meningitidis} traditionally has been considered strongly in the differential diagnosis of patients with adrenal hemorrhage and meningitis, while interstitial pneumonitis and myocarditis usually are not thought of as being caused by this infectious agent.13,27

By using immunohistochemical analysis and PCR, we were able to demonstrate \textit{N meningitidis} in the lungs of patients with interstitial pneumonitis. Clinicoradiographic reports have associated focal meningococcal pneumonia with \textit{N meningitidis} serogroup Y.25 This is in contrast with the cases of diffuse pneumonitis in our study, most of which were caused by serogroup C, and only 1 patient was found to have serogroup Y \textit{N meningitidis}. The histopathologic features in our cases resembled what is seen classically in ‘‘viral pneumonia,’’ with a generalized, diffuse, increased inflammation in the alveolar septa and with intra-alveolar edema. Focal
intra-alveolar inflammatory exudates characteristic of bacterial pneumonia were absent in the patients in the present series. The immunohistochemical assays of specimens from our patients revealed that the meningococci and meningococcal antigens were located predominantly in interstitial capillaries of the lung and not in the alveoli, suggesting that the interstitial inflammation was secondary to the bacteremic spread of meningococci.

Myocarditis has been described in a high proportion of fatal cases with meningococcaemia. The focal nature of the neutrophilic inflammatory infiltrate present in the myocardium of our patients was similar to that encountered in patients with systemic infections accompanied by myocardial inflammation, such as Rocky Mountain spotted fever. In our cases, the inflammatory infiltrates in the heart always were associated with immunohistochemical evidence of meningococci and meningococcal antigens, suggesting that the inflammation was related directly to meningococcal infection of the myocardium.

WFS is characterized by septicemia, shock, cutaneous petechiae and/or purpura, and adrenocortical insufficiency. Although WFS classically is associated with meningococcaemia, other infectious agents, such as H. influenzae and S. pneumoniae, can cause a similar clinical and pathologic picture. Hemorrhage and necrosis of the adrenal cortex are the cause of adrenocortical insufficiency, while cutaneous manifestations have been attributed to leukocytoclastic vasculitis. Finding meningococci in adrenal glands and in skin samples has proven difficult with the use of special stains. By using the immunohistochemical assay, meningococcal antigens were detected frequently in adrenal glands and skin blood vessels, suggesting that meningococcal fragments are important in the pathogenesis of adrenal hemorrhage and cutaneous manifestations.

During a 5-year study in a metropolitan area, clinical meningitis was present in up to 32% of adult patients with sporadic meningococcal disease. In our study, a large number of patients had immunohistochemical evidence of meningococci circulating in blood vessels of the central nervous system, while histopathologic evidence of meningitis was present in 50% (6/12). The mechanism by which meningococci cross the blood-brain barrier and cause meningitis is not completely understood. Our immunohistochemical study demonstrated a high concentration of N. meningitidis in blood vessels of the choroid plexus, suggesting that meningococci cross the blood-brain barrier at this site. In addition, thrombosis of choroid plexus blood vessels might have an important role by slowing blood flow and allowing invasion of the CSF space by meningococci. N. meningitidis in the meninges was seen only in the cases with histopathologic evidence of meningial inflammation. The propensity of N. meningitidis to invade meninges in some patients but not others is not well understood but might be due to host or bacterial virulence factors, the amount of meningococci in the bloodstream, or the disease time course.

The clinical picture of fulminant meningococcal disease can be confused with a variety of diseases that can cause shock in healthy individuals. The differential diagnosis might range from infections to behavioral diseases caused by substance abuse. In our study, we were able to identify meningococcaemia by using immunohistochemical analysis and real-time PCR when toxic shock syndrome, Chagas myocarditis, and cellulitis were the clinical diagnoses. Immunohistochemical analysis performed on formalin-fixed, paraffin-embedded tissue samples offers several advantages, including a specific diagnosis for N. meningitidis, preservation of tissue morphologic features (permitting localization of meningococci in specific cells), providing a permanent record, use of archival paraffin blocks, and use of specimens that pose minimal biohazard for laboratory personnel handling them. Owing to the cross-reactive nature of our polyclonal screening antibody with other Neisseria species, it is indispensable for confirming the diagnosis using other methods, such as PCR. In this study, real-time PCR confirmed the presence of N. meningitidis in formalin-fixed tissue samples, and serogroup determination was possible in a large majority of cases. Immunohistochemical analysis and PCR confirmatory testing of formalin-fixed tissue samples is available at the CDC through the Infectious Disease Pathology Activity. However, because meningococcal disease is life threatening, treatment should not wait for laboratory results. In addition, state health departments need to be notified because of public health importance.

Immunohistochemical and PCR assays performed on formalin-fixed tissue specimens can be used for diagnosis of N. meningitidis. This may prove useful in cases of fulminant disease for which culture samples were not obtained because there was little time for the patient to seek medical treatment or when cultures resulted in no growth because the patient had received previous treatment with antimicrobials. Immunohistochemical analysis is advantageous because it permits the visualization of meningococci and meningococcal antigens while preserving morphologic features, and it enables a better understanding of how N. meningitidis causes disease. Real-time PCR is advantageous because it permits determination of the N. meningitidis serogroup.
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References