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Postmortem Diagnosis of Meningococcemia by Detection of Capsular Polysaccharides

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ABSTRACT: Detection of specific meningococcal capsular polysaccharide (CPS) in postmortem blood permits rapid diagnosis of meningococcemia and differentiation from pneumococcemia and septicemia caused by *Haemophilus influenzae* Type b. We present studies validating application of latex agglutination assay for CPS on blood samples collected at autopsy, delineate the circumstances when CPS testing is indicated, and illustrate the usefulness of this procedure by several recent cases. Blood samples from victims dying of injury or disease other than infection were examined to determine whether the postmortem interval, bacterial contamination, anticoagulants, or delay in testing would result in false positive assays. Series 1 samples, collected so as to minimize bacterial contamination, were immediately submitted for assay. Series 2 evaluated the effect of adverse conditions of collection, anticoagulation, and prolonged sample storage. Despite extended postmortem intervals of up to 14 days, heavy bacterial contamination, prolonged storage at 4°C, deep hemolysis, and presence of anticoagulants, false positive assays were seldom observed.

KEYWORDS: pathology and biology, meningococcemia, capsular polysaccharides, postmortem examinations, bacterial antigen testing

Recognition of threats to public health is an important responsibility of medicolegal offices; one such threat is disease caused by *Neisseria meningitidis.* This microorganism can rapidly colonize the nasopharyngeal mucosa of contacts, and nonimmune and immunocompromised individuals are at increased risk of developing invasive disease which is often fulminant and fatal [*1*]. Rapid and accurate diagnosis is important because effective prophylactic therapy is available. When antemortem signs and gross autopsy observations indicate that a sudden, unexpected death may be due to meningococcal infection, establishing a definite diagnosis becomes a matter of extreme urgency. On the one hand, awaiting the result of a blood culture can result in potentially serious delay, since the culture may be negative because of (a) the fragile nature of the meningococcus, (b) overgrowth of contaminating microorganisms, or (c) antemortem administration of antibiotics. On the other hand, alarm-

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ing the contacts and community by reporting a case of meningococcal disease which ultimately is shown to be caused by some other microorganism is to be avoided. In such a setting, demonstration of a specific capsular polysaccharide (CPS) can establish the diagnosis as meningococcemia, pneumococcemia, or septicemia caused by *Haemophilus influenzae* [2-8].

CPS can be detected by a variety of methods including counter-immunoelectrophoresis (CIE) [2-5] and latex agglutination [6-8]. With CIE, under the influence of an electrical current, polysaccharide migrates towards the anode while electro-endosmotic flow carries antibody toward the cathode and polysaccharide; a line of precipitation occurs at the equivalence point. With latex agglutination, latex particles coated with antibody agglutinate in the presence of appropriate antigen. The latex technique is inexpensive and rapid, and the reagent reacts with some electrically neutral CPSs which are not detected by CIE.

CPS detection has not been applied previously to death investigation. In this paper we report studies validating latex agglutination tests for detection of CPS in autopsy blood samples by demonstrating that the postmortem interval, bacterial contamination, presence of anticoagulants, and storage of specimens at 4°C do not result in false positive assays. We also delineate the circumstances when assay for CPS is indicated, and illustrate the usefulness of the CPS assay by presenting five recent cases.

Methods

Specimen Collection

To determine whether the postmortem state adversely affects latex agglutination assays for CPSs, we examined autopsy blood samples from cases in which death was caused by injury or disease other than infection. The autopsies were performed at the Cuyahoga County Coroner's Office.

Series 1—The first series of cases evaluated the incidence of false positive CPS assays on postmortem blood when samples were collected so as to minimize bacterial contamination. Using sterile technique, we collected aliquots of heart blood without anticoagulant and immediately submitted them for assay. When the central portion of the cardiovascular system was partially or totally disrupted, blood was obtained from a thoracic cavity. An additional 6- to 10-mL aliquot of blood was divided equally between Bactec NR6A and NR7A blood culture vials. These cultures permitted determination of whether or not a latex agglutination result was a false positive, and if so, which bacterial contaminants could be responsible.

Series 2—The second series evaluated the effect on CPS assays of adverse conditions of collection, anticoagulation, and sample storage. Specimens consisted of plasma from postmortem blood submitted for toxicologic analysis. These samples, collected at the time of autopsy without sterile precautions, were placed in 50-mL test tubes containing 100-mg potassium oxalate and 125-mg sodium fluoride and subsequently refrigerated at 4°C until submitted for CPS assay.

Specimen Analysis

Blood samples for CPS testing were centrifuged and the serum or plasma separated; if testing was delayed, the samples were stored at −20°C. Latex agglutination tests for *N. meningitidis* (polyvalent reagent for Groups A, C, Y, and W135, and monovalent reagent for Group B), *Streptococcus pneumoniae* (omnivalent reagent), and *Haemophilus influenzae,* Type b were performed using Wellcome Diagnostics reagents according to the manufacturer's instructions.

Blood cultures were examined daily for seven days on a Bactec NR660 instrument (John-

ston Laboratories). Positive vials were subcultured and the isolates identified as to genus, species, or group.

Record Review

The gross anatomic autopsy observations indicating the need for CPS assay were delineated by reviewing cases of the Waterhouse-Friderichsen syndrome (WFS) and meningococcemia studied at the Cuyahoga County Coroner's Office. When indicated, hospital charts were examined to supplement the Coroner's records.

Results

Validation of Postmortem Polysaccharide Analysis

Causes of deaths of cases assayed for CPS are listed in Table 1. The estimated postmortem interval (EPMI), the time from discovery or pronouncement of death to autopsy, averaged 15.2 h (Range 2 to 30) for Series 1 and 19.9 h (Range 2 to 92) for Series 2. The latter group includes one case in which the body was in a state of early decomposition. Series 2 blood samples, in addition to the EPMI, were refrigerated at 4°C for an average of 12 days (Range 10 to 14) before being tested for CPS.

The results of tests for CPS are listed in Table 2. Forty-five (96%) of Series 1 and all 22 of Series 2 were nonreactive for CPS of *N. meningitidis*, Groups A, B, C, Y, and W135, *S. pneumoniae*, and *H. influenzae*, Type b. Only two sera from Series 1 were positive. One serum was falsely positive for *S. pneumoniae* CPS. The victim, a 2-year-old child, sustained immediately fatal shotgun injuries to the trunk. Blood cultures were negative for *S. pneumoniae* but positive for Enterobacteriaceae, viridans streptococci, *Staphylococcus aureus*, nonhemolytic streptococci, and *Clostridium* sp. A sample from a 19-year-old woman with Mixed Connective Tissue Disease produced positive reactions with 2 of the latex reagents (polyva-

TABLE 1—*Causes of deaths in autopsies from which blood for capsular polysaccharide assay was obtained.*

Cause of Death	Series 1[a]	Series 2[b]
Natural		
heart disease	8	11
seizure disorder	2	1
mixed connective tissue disease	1	1
bronchial asthma		1
berry aneurysm	1	
carcinoma	1	
cirrhosis		1
Violence		
blunt-force injuries	14	1
firearms	8	
poisoning	4	4
burns	4	
asphyxia	2	1
electrocution	1	1
stab wound	1	
Totals	47	22

[a] Blood samples immediately submitted for polysaccharide assay of serum.

[b] Anticoagulated samples refrigerated for 10 to 14 days before being submitted for polysaccharide assay of plasma.

TABLE 2—*Capsular polysaccharide assay.*[a]

Series	Number, %		
	Positive	Negative	Uninterpretable
1	1 (2)[b]	45 (96)	1 (2)[c]
2	0 (0)	22 (100)	0 (0)

[a]For CPS of *N. meningitidis* Groups A, B, C, Y and W135, *S. pneumoniae,* and *H. influenzae,* Type b.

[b]Positive for *S. pneumoniae.* COD: Shotgun wound of anterior trunk. Blood cultures negative for *S. pneumoniae* but yielded multiple contaminants.

[c]Serum sample uninterpretable due to positive reaction with *H. influenzae,* Type b and *N. meningitidis,* Groups A, C, Y and W135. COD: Mixed Connective Tissue Disease with polyclonal gammopathy and high titer of rheumatoid factor. Blood culture yielded multiple contaminants.

lent *N. meningitidis* and *H. influenzae,* Type b). Clinical studies had documented a polyclonal gammopathy and high titer of rheumatoid factor, the probable cause of the uninterpretable reactions.

Series 1 blood culture results are listed in Table 3. No growth was found in 6, and 94 isolates were obtained from the remaining 41 cultures. In 13 cultures, a single species was obtained including 1 isolate of *S. pneumoniae.* No source for this microorganism was demonstrated at autopsy, and the CPS assay was negative. Anamnestic data (known drug abuse), perivenous scars ("tracks"), pulmonary foreign body granulomata, and toxicologic analysis established the cause of death in this case as acute intoxication by combined effects of morphine and methadone.

Indications to Assay for Capsular Polysaccharide

From 1953 through 1986, the Cuyahoga County Coroner's Office performed nearly 50 000 complete autopsies, including 71 cases of the WFS and one case of meningococcemia without adrenal hemorrhage. The age, number, race, and sex of victims and etiologic agent of the WFS are listed in Table 4. Of the victims, 47 (66.2%) were children aged 1 to 10, with the largest group represented by white male infants less than 1 year of age. Of the decedents, 28

TABLE 3—*Blood culture isolates: Series 1.*[a]

Microorganism	No.	Microorganism	No.
Enterobacteriaceae[b]	26	*E. coli*	2
Strep. viridans group	25	*Bacillus* sp.	2
Clostridium sp.	10	Beta hemolytic strep.	2
Coag. neg. staphylococci	7	Enterococcus	1
Beta strep., Group B	4	*Strep. bovis*	1
Anerobic gm. neg. bacilli	4	*Corynebacterium* sp.	1
Non-ferment. gm. neg. bacilli	4	*Strep. pneumoniae*	1
Staphylococcus aureus	3	Yeast	1

[a]Ninety-four isolates from 47 patients.

[b]Enterobacteriaceae indicates species other than *E. coli,* (which were excluded by spot indol tests).

TABLE 4—*Analysis of 71 cases of Waterhouse-Friderichsen Syndrome, Cuyahoga County Coroner's Office, 1953-1986.*

Age, Years	No.	Race		Sex		Etiology[a]			Unknown
		W	B	M	F	*N. men.*	*S. pneu.*	*H. influ.*	
<1	23	20	3	16	7	9	0	1	13
1-10	24	18	6	13	11	14[b]	3[b]	2	6
11-20	5	5	0	3	2	3	1	0	1
20-40[c]	11	6	4	6	5[c]	3[d]	4	1	3
40-60	6	5	1	4	2	4[d]	0	0	2
>60	2	2	0	0	2	2	0	0	0
Total	71	56	14	42	29	35	8	4	25

[a]Five cases meningococcus isolated only from CSF.
[b]Meningococcus and pneumococcus isolated from blood and CSF.
[c]One decedent Hispanic.
[d]One case capsular polysaccharide of *N. meningitidis* detected. Blood culture not performed.

(39%) were dead on arrival, (DOA) and an additional 27 (38%) died less than 4 h after arriving at an emergency room. The etiologic agent was identified in 46 (64.8%). Of the positive cultures, 35 (76.1%) were *N. meningitidis*, 8 (17.4%) *S. pneumoniae*, and 4 (8.7%) *H. influenzae*. In 1 instance, *N. meningitidis* and *S. pneumoniae* were both isolated from blood and cerebrospinal fluid, and in 5 cases, *N. meningitidis* was isolated only from cerebrospinal fluid. The etiologic agent was not identified in 25 cases (35.2%) (neither ante- nor post-mortem culture obtained = 12; postmortem culture no growth = 6; postmortem culture overgrown by contaminants = 7).

The majority of victims with the WFS demonstrated a panoply of anatomical findings, including deep cyanosis, blotchy erythematous rash, petechial to purpuric eruption involving skin, conjunctivae, serosal, and mucosal surfaces, and bilateral adrenal cortical hemorrhages. Cutaneous manifestations (cyanosis, rash, and petechiae) may be entirely absent, and particularly in black victims, difficult to observe; the inferior palpebral conjunctivae are one of the better sites to observe petechiae [*1*]. Adrenal hemorrhage is a necessary component of the pathologic WFS, but may be focal and observed only microscopically [*9,10*]. The one fatality as a result of meningococcemia without associated macroscopic or microscopic adrenal hemorrhage was in a 33-year-old white woman who presented with intense cyanosis, multiple petechiae of the inferior palpebral conjunctivae, and numerous petechiae of serosal and mucosal surfaces. An antemortem blood culture was positive for *N. meningitidis*. Fatalities caused by meningococcemia without adrenal hemorrhage have been reported [*9,10*]; other indicia of meningococcemia have usually been present.

Asplenia and hemoglobinopathy have been associated with overwhelming sepsis and the WFS [*11-15*]. Table 5 lists the clinical diagnoses, age, race, and sex of the victims, status of spleen, and culture results. Encapsulated microorganisms are most commonly isolated (*S. pneumoniae, H. influenzae* and *N. meningitidis*), but a variety of other bacteria have also been implicated [*16,17*]. In eleven (15.5%) of our cases of WFS, either the spleen was absent or the patient had a hemoglobinopathy.

Discussion

CPS of an invading encapsulated microorganism accumulates in body fluids and can be detected by a variety of techniques including precipitin tube [*18-20*], CIE [*2-5*], and latex agglutination [*6,7*]. The latter procedure is simple, rapid, and more sensitive than CIE [*8,21*].

TABLE 5—*Cases of WFS with splenic atrophy, asplenia, or hemoglobinopathy.*

Clinical Diagnosis	Age	Race	Sex	Status of Spleen	Etiology
Sickle-cell disease	2	B	F	congested	*S. pneumoniae*
Congenital absence	2	W	F	aplasia	unknown
Sickle-cell disease	5	B	M	abnormal[a]	*S. pneumoniae*
Unknown	6	W	M	absent	*H. influenzae*
Spherocytosis, congenital	17	W	F	absent[b]	*S. pneumoniae*
Unknown	23	W	F	absent	unknown
Trauma	23	W	M	absent	unknown
Unknown	25	W	M	absent	*S. pneumoniae*
Sickle-cell disease	25	B	M	atrophic[c]	*H. influenzae*
Hodgkin's Disease	30	W	F	absent	*S. pneumoniae*
Hodgkin's Disease	36	W	M	absent	*S. pneumoniae*

[a] Siderotic nodules.
[b] Accessory spleen 30 gm.
[c] Weight of spleen 40 gm.

The assays are useful in clinical meningitis because detection of the CPS of a particular bacterium may permit initiation of specific rather than broad spectrum antibiotic therapy. However, difficulty can arise because of false negative and false positive results [*5–8,21*].

False negative results occur because the concentration of CPS is below the sensitivity of the assay (early infection), a prozone reaction, or loss of CPS antigenicity. Prozone reactions can be avoided by appropriate sample dilution. Loss of antigenicity can occur because of enzymatic destruction. The majority of the CPSs are stable, but that of *N. meningitidis,* Group B (NMGB), a polymer of $\alpha(2\rightarrow8)$ linked *N*-acetyl neuraminic acid, is degraded by neuraminidase [*22*] and is unstable even when stored at −20°C.[4] Loss of antigenicity can be circumvented by prompt assay.

False positive reactions occur because of the presence of nonspecific agglutinins and cross-reactivity of antisera with components of other bacteria. Most nonspecific agglutinins can be removed by heat [*7,23*]; for serum and plasma, this has the additional advantage of simultaneously deproteinizing the sample and liberating polysaccharide from antibody complexes. Cross-reactivity represents a more significant problem and has been reported in association with the CPSs of *N. meningitidis* [*24–26*], *S. pneumoniae* [*27–29*], and *H. influenzae,* Type b [*30,31*].

The most significant cross-reaction occurs between CPSs of NMGB and *E. coli,* particularly *E. coli* K1. The latter microorganism is responsible for upward of 80% of neonatal meningitis and 36% of neonatal sepsis [*32,33*]. Pyelonephritis, pneumonia, and meningitis have been reported in adults [*34–36*]. The CPSs of NMGB and *E. coli* K1, the major virulence factor in these pathogens, are chemically and immunologically identical [*26,37*]. This polysaccharide is a poor immunogen, perhaps because of "antigenic mimicry." Its glycosidic linkage is the same as that of the terminal residues of gangliosides and some neural glycoproteins [*38,39*].

These problems have been partially resolved by development of high titer, murine, monoclonal antisera [*40–42*]. Interpretation of positive latex agglutination reactions using these reagents varies, depending on the source of the sample. If obtained from a neonate, a positive reaction is presumed to reflect infection by *E. coli* K1, and if from an older subject, infection by *N. meningitidis,* Group B. Similar considerations would apply to the interpreta-

[4] "Latex Test to Detect *Neisseria meningitidis* Group B and *Escherichia coli* K1 Antigens," Wellcogen product literature, Feb. 1986, pp. 10–11.

tion of a positive postmortem CPS assay providing *E. coli* infections are excluded, for example, pyelonephritis, pneumonitis, and meningitis.

Although Edwards [*43*] detected CPS of Group C meningococci in postmortem blood of three patients dying from meningococcemia, this observation has not been applied to death investigation. Postmortem conditions are markedly different from the clinical setting. Autolytic and putrefactive processes commence shortly after death and become more pronounced with lengthening of the postmortem interval. Autolysis results from release of hydrolytic lysosomal enzymes while putrefaction is a more variable process, dependent upon host and environmental factors and accompanied by proliferation of a variety of invading colonic flora, resulting in progressive tissue destruction. Despite the complexity of these processes, our results demonstrate that extended postmortem intervals, heavy bacterial contamination, deep hemolysis, and prolonged sample storage at 4°C do not result in a significant number of false positive latex CPS assays.

In some respects, the postmortem state facilitates testing and interpretation of CPS assay. The blood of victims who die from overwhelming infection by encapsulated bacteria is likely to contain a quantity of CPS well within the range of assay sensitivity. Moreover, most CPS are stable and able to resist heat, drying, freezing, thawing, and prolonged refrigeration.

The most noteworthy advantage of the postmortem setting is the multiplicity of ancillary data available to assist in interpretation of the CPS assay. These include anamnestic information, findings of gross and microscopic anatomic examinations, and results of toxicologic analysis. Thus, in a setting of the WFS, detection of CPS of *N. meningitis* assumes far greater significance, even in the absence of a positive blood culture.

Case Reports

The following cases are illustrative of the utility of CPS analysis.

Case One

Previously in good health, a 58-year-old white man was found unresponsive and pronounced dead 47 min later. Autopsy was begun 24 h postmortem. Significant findings included prominent cyanosis, a blotchy erythematous rash involving head, trunk and extremities, focal and confluent dermal, conjunctival and serosal petechiae, and bilateral adrenal cortical hemorrhages. Postmortem blood cultures were unsatisfactory because of contamination, but plasma from the toxicologic blood sample, under refrigeration for 2 days, was positive for CPS of *N. meningitidis*, Group C.

Comment—Despite unsatisfactory postmortem blood cultures, a diagnosis of meningococcemia was made because of postmortem anatomic findings and positive assay for CPS of *N. meningitidis*, Group C.

Case Two

A 36-year-old white man was pronounced dead 1 h after being brought to an emergency room in "toxic shock." Past medical history included a staging laparotomy and splenectomy for Hodgkin's Disease. Autopsy was begun 9 h postmortem. Significant findings included blotchy erythematous rash, a few petechiae, surgical absence of the spleen, and bilateral adrenal cortical hemorrhages. Postmortem blood was positive for pneumococcal CPS, and cultures were positive for *S. pneumoniae.*

Comment—The cause for the WFS in this victim was established shortly after completion of the autopsy by detection of CPS of *S. pneumoniae.* It was unnecessary to alarm the community by reporting a possible case of meningococcemia.

Case Three

Eight hours after complaining of abdominal pain, a 28-year-old black man was found dead. At autopsy, begun 24 h after discovery, the only abnormal findings were bilateral pleural effusions. Microscopic examination revealed increased numbers of neutrophils in the small blood vessels of the heart and liver, and bilateral, focal, adrenal cortical hemorrhages. Postmortem plasma from the toxicologic blood sample, under refrigeration for 2 days, was reactive with polyvalent latex reagent for CPSs of *N. meningitidis.* A blood culture had not been performed.

Comment—The WFS was established only after microscopic examination revealed focal adrenal hemorrhages. No blood culture had been obtained, but analysis of plasma from the toxicologic blood sample was positive for CPS of *N. meningitidis.* A diagnosis of meningococcemia was made, and both the decedent's family and health officials were informed of the diagnosis.

Case Four

A 25-year-old black man with known homozygous sickle-cell disease died 7 h after being admitted with clinical diagnoses of sickle-cell crisis and drug overdose. Autopsy was performed 12 h postmortem. Significant findings included splenic atrophy (40 g) with siderotic nodules, and bilateral adrenal cortical hemorrhages. Antemortem blood culture and CPS assay of plasma from the postmortem toxicologic blood sample were positive for *H. influenzae,* Type b.

Comment—Detection of CPS of *H. influenzae,* Type b in the blood of this victim established the cause of the WFS before the blood culture became positive. Although the pneumococcus more commonly causes overwhelming fatal infection in patients with sickle-cell disease, septicemia with *H. influenzae* has been previously reported [*8*].

Case Five

A seven-month-old black female infant was seen in an emergency room the day before death because of fever of 105.4°F and vomiting. The baby was found expired the next morning. Autopsy was begun 24 h postmortem. Significant findings included scattered petechiae in the inferior palpebral conjunctivae and bilateral adrenal cortical hemorrhages. Postmortem serum was reactive with polyvalent reagent to *N. meningitidis; N. meningitidis* Type W135 was isolated from the blood culture.

Comment—Immediately after the autopsy was completed, blood was assayed and found reactive with polyvalent CPS meningococcal reagent. The known positive CPS assay alerted technologists to the possibility of a positive blood culture.

Summary

Recognition that a sudden death has been caused by *N. meningitidis* is important because the intimate contacts of the victim, particularly infants and young children, are at increased risk of developing invasive disease which may be fulminant and fatal.

The anatomic findings in meningococcemia are those of the Waterhouse-Friderichsen syndrome, that is, deep cyanosis, blotchy erythematous rash, petechial to purpuric eruption involving skin, conjunctivae, serosal and mucosal surfaces, and bilateral adrenal cortical hemorrhages. In some victims, particularly those who are black, the foregoing manifestations may be few and easily overlooked. Adrenal hemorrhages may be microscopic.

Because the Waterhouse-Friderichsen syndrome is nonspecific with respect to etiology, definitive diagnosis requires isolation and identification of the inciting agent. Capsular poly-

saccharide assay is *not* a substitute for blood culture; nevertheless, testing postmortem blood for specific capsular polysaccharide enables prompt presumptive recognition of the pathogen: *N. meningitidis, S. pneumoniae* or *H. influenzae,* Type b. When the anatomic findings of the Waterhouse-Friderichsen syndrome and capsular polysaccharide for *N. meningitidis* are simultaneously present, a diagnosis of meningococcemia is made and the attending physician and health authorities notified of the cause of death.

False negative results, because the concentration of capsular polysaccharide falls below the sensitivity of the assay, seem unlikely postmortem when death has been caused by overwhelming infection. False positive reactions can occur, but in our study were not a problem despite delay in testing, heavy bacterial contamination, prolonged storage at 4°C, deep hemolysis, and the presence of anticoagulants.

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