12. **Lambert-Zechovsky N, Bingen E, Guihaire E, Nancy C, Mercier JC, Beaufils F:** Choix d'une association bêta-lactamines-aminosides rapidement bactéricide dans le traitement des infections à *Pseudomonas aeruginosa* en réanimation infantile. Pathologie et Biologie 1986, 34: 855–858.
13. **Thabaut A, Meyran M:** Activité bactéricide comparée de l'imipénème et d'autres antibiotiques sur *Pseudomonas aeruginosa*. Pathologie et Biologie 1988, 36: 472–476.
14. **Drugeon HB, Courtieu AL:** Comparaison des pouvoirs bactéricides de 4 aminosides: amikacine, gentamicine, kanamycine et tobramycine. Annales de Microbiologie 1978, 130 A: 331–343.
15. **Chalkley LJ, Koornhof HJ:** Antimicrobial activity of ciprofloxacin against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* determined by the killing curve method: antibiotic comparisons and synergistic interactions. Antimicrobial Agents and Chemotherapy 1985, 28: 331–342.
16. **Thabaut A, Meyran M:** Activité bactéricide des nouvelles quinolones seules et en association. In: Pocidalo JJ, Vachon F, Régnier B (ed): Les nouvelles quinolones. Arnette, Paris, 1985, p. 45–71.
17. **Delisle-Mizon F, Lesage D, Denis C, Vergez P, Daguet GL:** Activité bactéricide comparée de la cefsulodine et de la ceftazidime sur *Pseudomonas aeruginosa*. Pathologie et Biologie 1983, 31: 383–386.
18. **Woolfrey BF, Gresser-Burns ME, Lally RT:** Ampicillin killing curve patterns of *Haemophilus influenzae* type b isolates by agar dilution plate count method. Antimicrobial Agents and Chemotherapy 1987, 31: 1711–1717.
19. **Woolfrey BF, Lally RT, Ederer MN, Gresser-Burns ME:** Oxacillin killing curves patterns of *Staphylococcus aureus* isolates by agar dilution plate count method. Antimicrobial Agents and Chemotherapy 1987, 31: 16–20.
20. **Perea EJ, Clavijo E, Nogales MC, Luque IG:** Interaction of aminoglycosides and cephalosporins against *Pseudomonas aeruginosa*. Correlation between interaction index and killing curve. Journal of Antimicrobial Chemotherapy 1988, 22: 175–183.
21. **Liebowitz LD, Saunders J, Chalkley LJ, Hendrik J:** In vitro selection of bacteria resistant to LY 146032, a new cyclic lipopeptide. Antimicrobial Agents and Chemotherapy 1988, 32: 24–26.
22. **Sykes RB, Bush K:** Interaction of new cephalosporins with beta-lactamases producing gram-negative bacilli. Reviews of Infectious Diseases 1983, 5: 356–366.
23. **Horodniceanu T, Derlot E, Cayeux P:** Mutants déficients à colonies naines de *Staphylococcus aureus*. Taux de mutation, fréquence de reversion. Compte Rendu à l'Académie des Sciences (Paris) 1974, 278: 161–163.
24. **Drugeon HB:** Réflexions sur les critères bactériologiques du choix d'une antibiothérapie pédiatrique. La Lettre de l'infectiologue 1988, 15: 542–544.
25. **Drugeon HB, Legallou F, Caillon J:** Méthodes d'étude de l'activité bactéricide. In: Courvalin P, Drugeon H, Flandrois JP, Goldstein F (ed): Bactéricidie. Aspects théoriques et thérapeutiques. Maloine, Paris, 1990, p. 113–126.

Role of Serological Tests in the Diagnosis of Immune Complex Disease in Infection of Ventriculoatrial Shunts for Hydrocephalus

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Seven cases of ventriculoatrial shunt infection with immune complex disease are reported in order to demonstrate the usefulness of measurement of levels of specific antibody to *Staphylococcus epidermidis* in diagnosis. Blood and cerebrospinal fluid cultures gave misleading results, and there was initial doubt about the diagnosis in all seven cases. All showed grossly elevated titres of antibody to *Staphylococcus epidermidis*, with raised serum C-reactive protein levels and depressed complement levels. Measurement of antibody to *Staphylococcus epidermidis* enables the diagnosis of chronic ventriculoatrial shunt infection to be made rapidly and reliably.

Six years after the first reports of bacterial colonisation of ventriculoatrial (VA) shunts for hydrocephalus, nephrotic syndrome was reported in two children with longstanding *Staphylococcus epidermidis* shunt colonisation (1) and this disease entity was later termed "shunt nephritis" (2). The condition is now known to be due to the deposition of immune complexes on the glomerular basement membranes, leading to activation of the cascade of immune phenomena which results in glomerular destruction (3–5). Despite the recommended use of serological screening in patients with VA shunts (6), which allows the infection to be diagnosed before immune complex disease appears, cases still occur and are reported in the literature. We report seven cases which illustrate the difficulties in clinical and laboratory diagnosis of the disease and the usefulness of serological tests.

Materials and Methods. The anti-*Staphylococcus epidermidis* titre (ASET) was determined using a

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previously described method (7) with minor modifications. Briefly, a stock strain of *Staphylococcus epidermidis* sensu stricto was autoclaved at 121 °C for one hour, and aliquots of a suspension were then diluted in buffer to give an A_{490} value of 0.890 for use. Serum samples were double-diluted in buffer in microtitre trays and antigen suspension was added to each well. Final serum dilutions ranged from 1/20 to 1/40,960. The trays were covered and refrigerated at 4 °C overnight and the endpoint read as the highest dilution which gave no sign of a "button". Positive and negative rabbit sera were included as controls. Normal values vary with age (7), and ideally a preoperative sample should be available as a baseline, but single results can be interpreted easily in cases with clearly elevated titres. Serum C-reactive protein (CRP) and complement levels (C3c and C4) were measured in the serum by rate immune turbidometry using a Turbitimer and specific antiserum (Behringer, UK). Details for the seven patients reported are shown in Table 1.

Results and Discussion. The findings are shown in Tables 1 and 2. The period from the most recent shunt operation to diagnosis ranged from 3 to 14 years (median of 5 years). The duration of illness severe enough to warrant medical attention ranged from 2 weeks to 13 years (median 3 months). Fever was the presenting feature in five patients, although two patients never experienced pyrexia. Headaches and malaise were common symptoms, and two patients had chills with one showing an intermittent rash. Three patients also had nausea and vomiting, and two had low back pain. Two patients (cases no. 3 and 4) were treated unsuccessfully for anaemia with iron supplementation before the correct diagnosis was made. Anaemia was a feature in all seven cases. The diagnosis was in doubt initially in all seven cases, but in cases no. 2, 3, 4 and 5 an incorrect diagnosis was first made and acted upon. In case no. 4, the persistent anaemia was thought to be due to blood loss, possibly from reflux oesophagitis, and a Nissen fundoplication was planned. The patient's anaemia resolved after treatment for shunt infection without the fundoplication. All except two cases showed some evidence of renal dysfunction.

The microbiological results are shown in Table 2. The incidence of negative or misleading blood cultures is evident, and cultures of cerebrospinal fluid (CSF) aspirated from the shunt reservoir also show negative results in most cases.

The serological results are shown in Table 2. Grossly raised ASET results were found in all seven cases, and all had raised CRP levels. Similarly, all had depressed levels of one or both of the complement fractions tested.

Case no. 3 serves to illustrate the diagnostic difficulties. The 6-year-old girl with spina bifida had had a VA shunt inserted soon after birth; the last revision was three years previously. She had been unwell for approximately one year with malaise and drowsiness, intermittent headaches with vomiting and occasional fever. She was investigated elsewhere, found to be anaemic and given a course of oral iron. She did not improve and was admitted for investigation. On admission she was pyrexial and unwell. Laboratory findings showed anaemia despite iron therapy. A CSF sample from the shunt reservoir and a blood culture showed no growth. Urinalysis showed red cells and albumen. Two further blood cultures then grew dissimilar strains of *Staphylococcus epidermidis*, further confusing the picture. The ASET was then 20480 (normal for this age 160). On removal the shunt grew *Staphylococcus epidermidis* with the same characteristics as one of the blood culture isolates.

The manifestations of infection in ventriculoatrial and ventriculoperitoneal shunts are very different. In ventriculoperitoneal shunts infections present almost always within six months of operation, the presenting features usually being those of shunt malfunction, with or without those of sepsis superimposed. In VA shunts the features of infection are very variable. Some cases present with obvious systemic sepsis soon after operation, but others present years later with evidence of chronic, low-grade bacteraemia. Although these cases have also been shown to have their origin at surgery (8, 9), patients usually show nonspecific or even frankly misleading symptoms in the intervening period, and inappropriate involvement of several other medical specialties sometimes leads to delay in diagnosis (10, 11).

Immune complex disease in patients with VA shunts is associated with chronic bacteraemia (2, 11), and can be prevented by early recognition and treatment of the infection (6). Due to the difficulty in making a clinical diagnosis before the condition becomes overt, there is greater reliance on laboratory tests. Blood culture is always carried out, but can be misleading due to falsely positive or contaminated cultures. No positive blood cultures at all were found in cases no. 1 and 7. Although few cultures were taken in these cases,

Table 1: Details on patients with immune complex disease and urinary findings.

Case no.	Sex	Age at diagnosis (years)	Cause of hydrocephalus	Time since last operation (years)	Fever	Anaemia	Urea	Creatinine	Urinalysis		
									RBC	Casts	Protein
1	F	23	aqueduct stenosis	8	–	+	R	R	+	+	+
2	F	37	aqueduct stenosis	7	+	+			–	–	–
3	F	6	spina bifida	3	+	+	ND	ND	+	–	+
4	F	24	spina bifida	5	–	+			+	+	+
5	M	16	post. fossa tumour	5	+	+			–	–	–
6	F	20	spina bifida	14	+	+	R	R	+	+	+
7	M	27	astrocytoma	5	+	+	R	R	+	+	+

RBC: red blood cells; R: raised; ND: not done; +: present; –: absent.

Table 2: Microbiological and serological findings in patients with immune complex disease.

Case no.	Blood culture	CSF culture	Shunt culture	ASET[c]	CRP[d] (mg/l)	C3c[d] (g/l)	C4[d] (g/l)
1	a) negative b) negative	*S. epidermidis*	negative[b]	10240 (640)	54.6	0.316	0.18
2	a) *S. epidermidis*[a] b) *S. epidermidis*[a]	negative	*S. epidermidis*[a]	10240 (640)	38.2	0.67	0.13
3	a) negative b) *S. epidermidis*[a] c) *S. epidermidis*	negative	*S. epidermidis*[a]	20480 (160)	36.0	0.31	0.15
4	a) *Acinetobacter* sp. + *S. epidermidis* b) negative c) negative d) negative e) negative f) negative g) *S. capitis*[a]	negative	*S. capitis*[a]	5120 (640)	32.0	0.255	0.07
5	a) negative b) *S. epidermidis*[a] c) negative d) *S. epidermidis* + *S. haemolyticus* e) *S. epidermidis*	not done	*S. epidermidis*[a]	2560 (320)	53.8	0.38	0.12
6	a) negative b) negative c) *S. epidermidis*[a] d) *S. epidermidis*[a]	a) negative b) negative c) *S. epidermidis*[a]	S. epidermidis[a]	> 40960 (640)	48.9	0.36	0.14
7	negative	*S. epidermidis*	negative[b]	5120 (640)	45.0	0.61	0.17

[a] Isolates indistinguishable within (but not between) cases.
[b] Antibiotics given for several days previously.
[c] Anti-*Staphylococcus epidermidis* titre; normal titre for age shown in brackets.
[d] Normal values: C-reactive protein ≤ 10 mg/l; complement fraction C3c 0.55-1.2 g/l; complement fraction C4 0.2-0.5 g/l.

where multiple cultures were done, as in cases no. 4, 5 and 6, this did not greatly assist in establishing a diagnosis. Overall, of 28 cultures taken, 14 were negative and only 9 of the remainder grew the shunt colonising strain.

Most cases of shunt colonisation are due to *Staphylococcus epidermidis*. Using the ASET test, the titre of antibody to this organism can be shown to rise in a predictable manner with age in uninfected patients, and to rise to very high levels in those with colonised VA shunts (6). The antigenic complex used in the ASET is common to most species of coagulase-negative staphylococci, and this is shown by the reaction to *Staphylococcus capitis* in case no. 4. The test can be used diagnostically but it can also be used as a screening test in the first six months after surgery (7). In our experience all cases of VA shunt colonisation are detectable serologically in this period, although it is possible that some may take longer to show seroconversion.

The CRP level is rarely raised in uncomplicated *Staphylococcus epidermidis* VA shunt colonisation, and a high CRP level found long after surgery is often indicative of immune complex disease although this must be confirmed by demonstration of depressed C3c and C4 fractions. Anaemia, refractory to iron therapy, is almost always a feature of VA shunt colonisation (2, 6), even in the absence of shunt nephritis, and is a clinically useful finding.

Colonisation due to *Staphylococcus epidermidis* can be diagnosed reliably in chronic cases using the ASET test and if this is done early, immune complex nephritis can be avoided (6).

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References

1. **Black JA, Challacombe DN, Ockenden BG:** Nephrotic syndrome associated with bacteraemia after shunt operations for hydrocephalus. Lancet 1965, ii: 921–924.
2. **Stauffer UG:** "Shunt nephritis": Diffuse glomerulonephritis complicating ventriculoatrial shunts. Developmental Medicine and Child Neurology 1970, 12, Supplement 22: 161–164.
3. **Caron C, Luneau C, Gervais MH, Plante GE, Sanchez G, Blain G:** La glomérulonéphrite de shunt: manifestations cliniques et histopathologiques. Canadian Medical Association Journal 1979, 120: 557–561.
4. **Dobrin RS, Day NK, Quie PG, Moore HL, Vernier RL, Michael AF, Fish AJ:** The role of complement, immunoglobulin and bacterial antigen in coagulase-negative staphylococcal shunt nephritis. American Journal of Medicine 1975, 59: 660–673.
5. **Wyatt RJ, Walsh JW, Holland NH:** Shunt nephritis. Role of the complement system in its pathogenesis and management. Journal of Neurosurgery 1981, 55: 99–107.
6. **Bayston R, Swinden J:** The aetiology and prevention of shunt nephritis. Zeitschrift für Kinderchirurgie 1979, 28: 377–384.
7. **Bayston R:** Serological surveillance of children with CSF shunting devices. Developmental Medicine and Child Neurology 1975, 35, Supplement 17, 104–110.
8. **Bayston R, Lari J:** A study of the sources of infection in colonised shunts. Developmental Medicine and Child Neurology 1974, 32: 16–22.
9. **Schoenbaum SC, Gardner P, Shillito J:** Infections of cerebrospinal fluid shunts: epidemiology, clinical manifestations and therapy. Journal of Infectious Diseases 1975, 131: 543–552.
10. **Stickler GB, Myung H, Shin MH, Burke EC, Holley KE, Miller RH, Segar WE:** Diffuse glomerulonephritis associated with infected ventriculoatrial shunt. New England Journal of Medicine 1968, 20: 1077–1082.
11. **Strife CF, McDonald BM, Ruley EJ, McAdams AJ, West CD:** Shunt nephritis: the nature of the serum cryoglobulins and their relation to the complement profile. Journal of Pediatrics 1976, 88: 403–413.

Comparison of Four Genotyping Assays for Epidemiological Study of Methicillin-Resistant *Staphylococcus aureus*

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Twenty-six methicillin-resistant *Staphylococcus aureus* strains were genetically differentiated by interrepeat PCR and the results compared with those of ribotyping, pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA analysis obtained in a previous study for the same strains. The comparison showed that the PCR-mediated assays were as discriminatory as PFGE, whereas ribotyping was the least powerful

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