Endocarditis caused by Haemophilus parainfluenzae identified by 16S ribosomal RNA sequencing

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Abstract
Haemophilus parainfluenzae, a human commensal, is an infrequent cause of serious disease. A case of endocarditis caused by this organism in a five year old boy with complex congenital heart disease is reported. The course of this disease was very aggressive, leading to heart failure, disseminated intravascular coagulation and multiorgan failure in spite of appropriate antibiotics and surgical intervention. The difficulties in the detection and identification of H parainfluenzae using conventional culture based technology, and the potential role of molecular techniques, are highlighted.

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Keywords: Haemophilus parainfluenzae; endocarditis.

Haemophilus species are members of the normal oropharyngeal flora in healthy people. The pathogenicity of species other than Haemophilus influenzae is low. Haemophilus species, predominantly H paraphrophilus, H parainfluenzae and H aphrophilus may account for 0.8-1.3% of all cases of infective endocarditis. Other reported infections caused by H parainfluenzae include septicaemia, brain abscess, respiratory, intra-abdominal, urogenital, and bone and joint infections; the most frequently described infections being infective endocarditis and meningitis. Here, we describe the clinical course, laboratory diagnosis and management of a case of infective endocarditis caused by H parainfluenzae.

Case report
A five year old boy presented in May 1995, with a three week history of malaise with fever and a 24 hour history of headache and vomiting. An upper respiratory tract infection, at the onset of his illness, had been treated empirically with a five day course of penicillin V. The patient had congenital cardiac anomalies consisting of transposition of great vessels, ventricular septal defect (VSD) and pulmonary valve stenosis. He had a palliative operation in April 1990 followed by a repair in January 1994. An episode of coagulase negative staphylococcal endocarditis in April 1994 necessitated the removal of the VSD patch and the aortic monocusp. The patient underwent further surgery in December 1994 to replace the aortic monocusp and the VSD patch and made an uneventful recovery.

On examination at this presentation, the patient was pyrexial and had mild clubbing but no splinter haemorrhages. A pre-existing grade four/six pansystolic murmur at the left sternal edge was present. There were no stigmata of infective endocarditis. Enlarged but non-inflamed tonsils were noted. Laboratory investigation revealed a white blood cell (WBC) count of 43.6 x 10^9/l with a neutrophilia, platelets 135 x 10^9/l, haemoglobin 12.3 g/dl and C reactive protein (CRP) 272 mg/l. Urine analysis was negative. A throat swab grew Streptococcus pyogenes. A transoesophageal echocardiogram showed vegetation on the VSD patch. The patient was treated empirically with intravenous vancomycin, benzyl penicillin and rifampicin. Three out of nine blood cultures taken before antibiotic treatment began grew a pleomorphic Gram negative bacillus after 48 hours' incubation. The isolate was presumptively identified as H parainfluenzae by its V factor dependency and biochemical reactions (api NH, bioMerieux, Marcy-l'Etoile, France). However, other phenotypic characteristics, such as colonial morphology and growth on MacConkey agar, were atypical. Comparative sequence analysis of 16S ribosomal RNA gene showed >99.7% homology with H parainfluenzae (fig 1). The isolate was resistant to amoxy-
cillin (non-beta-lactamase mediated) but was sensitive to cefotaxime, gentamicin, ciprofloxacin, and rifampicin by Stokes method. Minimum inhibitory concentrations and tests for synergy could not be satisfactorily determined because of poor growth of the organism. Antibiotic therapy was changed to a combination of cefotaxime, rifampicin and gentamicin. However, the patient remained pyrexial and later deteriorated further, developing a coagulopathy and signs of heart failure. On day 17, he underwent emergency cardiac exploration, during which he suffered a brief period of cardiac arrest. Large vegetations were found around the dehisced VSD patch, which was excised, and the edge was debrided. There was no growth from this material, nor from several further blood cultures. Postoperatively, the patient required mechanical ventilation and inotropic support. After initially improving, his condition deteriorated again, with development of multiorgan failure. As a result of deteriorating liver and kidney functions, gentamicin and rifampicin were stopped and from day 28, antibiotic therapy was continued with cefotaxime and ciprofloxacin. Because of persistent signs of sepsis, teicoplanin, and later vanco-

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mycin and liposomal amphotericin B, were administered empirically, although secondary bacterial or fungal infection was never demonstrated. On day 38, the patient underwent pulmonary artery banding in order to reduce pulmonary blood flow, and on day 60, the VSD was repaired. His general condition began to improve from day 51 onwards; ciprofloxacin and cefotaxime were withdrawn after a total period of eight and five weeks, respectively. The patient’s WBC count returned to normal levels and there was gradual reduction in the CRP concentration to 5 mg/l by the 16th week of admission. However, he was left with a mild right sided hemiparesis of the lower leg and impairment of speech. The patient was discharged home 18 weeks after admission and at follow up 11 weeks later, his cardiac function was comparable with prior to this illness. There has not been a recurrence of the infection five months following discharge.

**Discussion**

*H parainfluenzae*, like other members of the HACEK (Haemophilus spp., Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, and Kingella kingae) group, is a fastidious organism, often difficult to isolate from blood culture and often difficult to identify. Our isolate grew from only three out of nine sets of blood cultures. Growth on the blood-medium interface in the form of “puff” balls is characteristic, and provided a clue as to the identity of our isolate. However, the identity could not be confidently confirmed by phenotypic methods. Identification of our isolate was confirmed by 16S ribosomal RNA sequencing. This showed >99% sequence homology with a reference strain of *H parainfluenzae* but was distinct from other *Haemophilus* species (fig 1). The usefulness of this technique has been reported previously by Hamed et al.1

Predispoding factors for the development of *H parainfluenzae* induced endocarditis include dental abscess, procedures or other oral trauma, upper respiratory tract infections, otitis media, and intravenous drug abuse. Our patient had a confirmed *S pyogenes* throat infection at presentation, which may have facilitated entry of *H parainfluenzae* into the blood stream. Underlying heart disease, as in our case, is present in only about 50% of the cases.3

The onset of endocarditis caused by *Haemophilus* species is frequently subacute.4 The clinical and bacteriological response to appropriate antibiotics is often slow.5 In our case, in spite of surgery, response in terms of clinical observations and laboratory parameters was not seen until after 40 days of appropriate antibiotic therapy. This led to empirical use of additional toxic and expensive antimicrobial agents. However, there was never any evidence of superadded infection, either from microbiological investigations or clinical response to introduction of the additional agents.

A 23% mortality and 77% morbidity (primarily resulting from valvular and embolic complications) was reported amongst 13 cases of *H parainfluenzae* endocarditis in children.2 Major arterial embolisation, especially of the cerebrovascular circulation, is reported to occur in 50–63% of cases of *H parainfluenzae* endocarditis. This is thought to be because of the friable nature of the vegetation. Cerebrovascular embolic events are often the leading cause of mortality and morbidity in these patients.

Although our patient developed neurological deficit following this infection, computed axial tomography scan of the brain was normal suggesting some other cause, such as cerebral anoxia during his intraoperative cardiac arrest.

The usual therapy for endocarditis due to this organism has been large doses of intravenous ampicillin, often combined with an aminoglycoside. There is less experience in treating ampicillin resistant strains. Therapy should be based on antibiotic susceptibility testing, although this is often unsatisfactory because of difficulty in growing the organism.6

Agents active against ampicillin resistant...
strains are third generation cephalosporins and ciprofloxacin. Surgical intervention in the form of vein bypass, valve replacement or embolec-
tomy is often necessary in cases of *H parainfluen-
zae* endocarditis.2,8

Awareness of *H parainfluenzae* as a cause of endocarditis is important because empirical
antibiotic therapy, especially that chosen for
post cardiac surgery cases, may not cover this
organism. Delay and difficulty in isolation and
identification of the organism is often experi-
cenced, leading to destruction of cardiac
structure. Our case also exemplifies that the
clinical response to appropriate antibiotic
therapy may be slow. Lack of response to
appropriate medical and surgical intervention
should not be automatically considered as an
indication of treatment failure or superadded
infection. Advances in our understanding of
the phylogenetic significance of 16S ribosomal
RNA and the development of PCR technology
offer opportunities for both the identification
and detection of the HACEK group of
organisms.

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ribosomal RNA sequencing.

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Primary extramedullary plasmacytoma of the liver

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Abstract

Extramedullary plasmacytoma of the liver is a rare tumour, only two cases of
which have been reported so far. A third
case arising in a 22 year old woman, who
presented with abdominal pain and en-
largement of the liver, is described. Ultra-
sound and a computed tomography scan
showed a solitary hepatic mass, 12 cm
diameter, involving both lobes of the liver.
Serum immunoelectrophoresis revealed an
IgG k monoclonal gammopathy. Histol-
ogically, the tumour was composed of
mature plasma cells with mild atypia. The
plasma cells infiltrated the liver paren-
chyma and showed k light chain restric-
tion. The monoclonal nature of the
tumour was also demonstrated by PCR
amplification of the immunoglobulin
heavy chain genes. There was no evidence
of bone involvement and repeated bone
marrow aspirates and biopsy specimens
were normal. The patient was treated with
eight courses of chemotherapy. One year
after diagnosis, the patient is well, the size
of the tumour has decreased and the para-
proteinaemia has disappeared.


Keywords: liver; extramedullary plasmacytoma.

Primary non-Hodgkin’s lymphomas of the liver are rare. Many of these lymphomas are high
grade, with only a minority of cases presenting as
low grade tumours. A proportion of primary low grade lymphomas of the liver would seem to be B cell lymphomas of mucosa
associated lymphoid tissue (MALT) type. To
our knowledge, only two cases of primary
extramedullary plasmacytoma of the liver has
been reported to date. Here, we present a
third case.

Case report
A 22 year old woman presented with a history of
abdominal pain of four months duration.
Physical examination was unremarkable except
for moderate enlargement of the liver. Ultra-
sonographic examination and a computed
tomography scan of the abdomen revealed a
solitary hepatic mass, 12 cm diameter, which
extended to both lobes. Haematological in-
dexes were within normal limits but the eryth-
bocyte sedimentation rate was 110 mm/hour.
The patient was positive for hepatitis B virus
(HBV) surface antigen but was negative for
antibodies against HBV surface antigen and
hepatitis C virus. Liver function tests were
within the normal limits. Serum protein
electrophoresis showed increased γ-globulins,
58.7% (normal range 9–20%). Serum immuno-
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