CASE REPORT

Non-typeable *Haemophilus influenzae* infective endocarditis in a renal transplant recipient: compromised host or virulent strain?

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SUMMARY

Non-typeable *Haemophilus influenzae* (NTHI) rarely cause endocarditis. Of the limited reports of *H influenzae* endocarditis, most have been due to encapsulated organisms or have had limited bacterial characterisation. We encountered a transplant recipient with native valve NTHI endocarditis and were intrigued to find no previous descriptions of this entity. Although it was tempting to ascribe this infection to our patient's immunocompromised status, we investigated his pathogen further and found that it displayed features common to invasive NTHI strains including gene expression for two IgA proteases and serum resistance. Multilocus sequence typing grouped our NTHI strain with MLST 159, a group associated with invasive NTHI infections. Our strain shared identical outer membrane protein P2 sequences and protein patterns with MLST 159 strains. Aside from providing the first characterisation of native valve NTHI infection, our investigation reveals features of epidemiologically unrelated, clonal NTHI strains that have a predilection for invasive infections.

BACKGROUND

Non-typeable *Haemophilus influenzae* (NTHI) is an exclusively human pathogen which is a common cause of sinopulmonary infections such as otitis media, exacerbations of chronic obstructive pulmonary disease, community acquired pneumonia, sinusitis and conjunctivitis. We encountered a renal transplant patient with native valve endocarditis due to NTHI and we were surprised to find no previous reports of this entity. Although he was immunocompromised, we found no reports of invasive NTHI disease in solid organ transplant patients. We hypothesised that our patient's strain of NTHI had specific virulence traits which, coupled with his immunosuppressed state, resulted in invasive infection. In addition to effectively treating him, we performed studies to better characterise his pathogen.

CASE PRESENTATION

A 64-year-old African-American male renal transplant patient presented to the Buffalo VA Medical Center with symptoms of fever and chills for 2 days. He received his transplant in 2004 for hypertensive nephropathy and took mycophenolate, tacrolimus and prednisone daily. He had no transplant-related complications and his serum

creatinine remained 1.4–2.3 mg/dL. There were no signs of specific infection and he initially received broad-spectrum antibiotics. His physical examination was remarkable for a temperature of 100.8°F and a new systolic murmur along the left sternal border. His white cell count was 6.3×10^3 cells/mm³, platelets were 53 000/mm³ and serum creatinine was 2.5 mg/dL. Urinalysis and urine culture were unremarkable. All other laboratory values were unremarkable. ECG, chest X-ray and CT of his head and sinuses were unrevealing. Two sets of blood cultures, drawn 29 h apart, grew small Gram-negative bacilli. Formal identification was performed by the New York State Department of Health. Real time PCR determined the absence of the *bexA* as well as *capB* genes, confirming the blood isolate to be NTHI that proved to be β -lactamase negative. A transthoracic echocardiogram detected independently mobile aortic valve echodensities, 0.7 cm $\times$ 0.3 cm in diameter.

INVESTIGATIONS

Identification of the bacterial strain as NTHI and not *H haemolyticus* was further confirmed by immunoblot analysis with monoclonal antibody 7F3, which detects outer membrane protein (OMP) P6, a well-conserved epitope of NTHI.¹ To determine if our NTHI strain shared features with other invasive NTHI strains, we investigated it for several properties. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) of whole cell lysates of our patient's strain and of NTHI 11P6H, a well-studied invasive NTHI strain, was performed (figure 1A). Analysis of our patient's strain (lane a) was identical to that of NTHI 11P6H (lane b) except for a single band. Clonally related NTHI strains that contain *igaA* as well as *igaB* genes, that encode for two distinct IgA proteases, appear particularly adapted to infect adults with chronic obstructive pulmonary disease (COPD).² Reverse transcriptase PCR determined that the genome of our patient's NTHI strain expressed genes for *igaA* as well as *igaB* (figure 1B).

Resistance to the killing effects of serum provides NTHI with a potential survival advantage.³ Serum susceptibility of our patient's NTHI strain was determined in two separate experiments, as previously detailed.³ Colony counts of each was performed in comparison to a negative control strain (NTHI7p49H), exhibiting 0% survival in each, and a positive control strain (NTHI101p3H) exhibiting

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Figure 1 Molecular analyses of non-typeable *Haemophilus influenzae* (NTHI) endocarditis strain. (A) Coomassie Blue stained sodium dodecyl sulfate polyacrylamide gel electrophoresis of whole cell lysates. Lane a: nontypeable *H. influenzae* strain 11P6H (a prototype multilocus sequence typing 159 strain); lane b: endocarditis patient NTHI strain. Molecular mass standards are noted on the left in kilodaltons. The arrow denotes a band that is present in strain 11P6H but absent in the patient isolate. (B) Ethidium bromide stained agarose gel of PCR reactions with genomic DNA of our patient's NTHI isolate (lanes a and b) and NTHI strain 11P6H (lanes c and d). PCR reactions used oligonucleotide primers.

57% and 100% survival in each experiment respectively. Our patient's NTHI strain had 100% survival in pooled donor serum in each experiment (figure 2).

Finally, to assess our strain in relation to other NTHI strains, multilocus sequence typing (MLST) was performed, by determination of defined internal fragments of seven housekeeping genes.⁴ Sequences submitted to <http://haemophilus.mlst.net> grouped our patient's NTHI strain in MLST 159, a group that is associated with invasive infections and infections in COPD.⁵ The gene that encodes NTHI OMP P2 has remarkable diversity of sequences among NTHI strains.⁶ Therefore, to investigate

Figure 2 Serum bactericidal assays. Serum survival of our patient's non-typeable *Haemophilus influenzae* (NTHI) endocarditis strain (A) was determined in two separate experiments in comparison to a negative control strain NTHI7p49H (B) and a positive control strain NTHI101p3H (C) in pooled donor serum.

the relationship between our NTHI strain and MLST 159 strains by a second independent means, we determined the sequence of the OMP P2 gene of our NTHI isolate and found it to be identical to the sequences of the other MLST 159 strains.

TREATMENT

Our patient received 6 weeks of ceftriaxone, 2 g intravenously each day with a complete resolution of presenting signs and symptoms.

OUTCOME AND FOLLOW-UP

One week following completion of antibiotics, two sets of blood cultures confirmed that the patient was no longer bacteraemic and was clinically cured. He remains well 9 months after completion of antibiotics.

DISCUSSION

This is the first well-characterised case of NTHI endocarditis. The usual spectrum of NTHI disease in humans consists of sino-pulmonary infections such as otitis media, exacerbations of COPD, community acquired pneumonia, sinusitis and conjunctivitis.⁷ Endocarditis due to *Haemophilus* species collectively comprises up to 5% of all infective endocarditis.⁷ Of these, the overwhelming majority is due to *Haemophilus parainfluenzae*, *Haemophilus aphrophilus* and *Haemophilus paraphrophilus*.⁷ *H. influenzae* endocarditis is exceedingly rare, comprising 0.8–1.3% of reported endocarditis cases.⁸ For example, in two series of endocarditis due to *Haemophilus* species, *H. influenzae* accounted for only 1 of 14 cases in the first and only 3 of 42 cases in the second.^{8,9} *H. influenzae* strain typing was not given for either of these series.

Review of the literature revealed only 15 reported cases of all *H. influenzae* endocarditis (table 1). Of these, five were due to encapsulated strains including several type b strains^{10–13} and one type a strain.¹³ Of the 15 cases, 9 gave no indication of serotyping and thus NTHI may have been among these, but

Table 1 Reported *Haemophilus influenzae* endocarditis cases

Reference	Serotype	Age/ Sex	Valvular involvement	Special features
8	Not given	76/M	Aortic, mitral, tricuspid	
	Not given	30/F	Tricuspid	
	Not given	66/F	Mitral	
9	Not given	25/F	Not given	
	Type b	2/F	Tricuspid	
	Not specified*	20/F	Not given†	
10	NTHI	9/F	Not given†	Intra-aortic Gortex graft
	Type b	29/F	Mitral	
	Type b	55/M	Aortic	
11	Type a	19/M	Mitral, aortic	Probable rheumatic heart disease
	Type b	17/F	Tricuspid	Rheumatic heart disease
	Not given	37/M	Aortic	Rheumatic heart disease
12	Not given	32/F	Mitral, aortic	Rheumatic heart disease
	Not given	25/M	Aortic	Rheumatic heart disease
	Not given	59/F	Mitral	Prosthetic valve
13	Not given	37/M	Aortic	
	Not given	32/F	Mitral, aortic	
	Not given	25/M	Aortic	
14	Not given	59/F	Mitral	
	Not given	59/F	Mitral	
	Not given	59/F	Mitral	

*Listed only as non-type b.

†No echocardiographic vegetations identified.

may have been unrecognised.^{8–10 13 14} One involved a prosthetic-valve infection.¹⁴

We found only one report of NTHI endocarditis and that involved prosthetic intravascular material.¹⁰ Early reports of endocarditis due to *H influenzae* strains whose serotyping was not reported may predate routine specific identification. A review of all reported cases of *H influenzae* endocarditis indicates a female predominance (2:1), equal distribution between aortic and mitral valves and a broad age range (2–76 years), consistent with demographics for other causes of endocarditis.¹⁵

Vaccination against encapsulated *H influenzae* (type b) with the conjugated Hib vaccine over the past 15–20 years has dramatically diminished the incidence of infection with this pathogen.¹⁶ At the same time, a relative rise in incidence of non-type b *H influenzae* strains has been noted in Western countries.¹⁷ In Sweden, where Hib vaccine coverage rate is 99%, a significant increase in the incidence of invasive NTHI disease has occurred. Among these, 37% were in individuals with evidence of immune compromise.¹⁸ Thus, the emergence of invasive NTHI strains, in healthy as well as immunocompromised patients in the Hib vaccine era, bears special significance.

The virulence traits of encapsulated strains have been well described, including a polysaccharide capsule, composed of polyribitol ribose phosphate for type b *H influenzae* that permits evasion of phagocytosis and opsonisation.⁷ In contrast, virulence traits of non-typeable strains are not well defined. A high degree of genomic diversity among non-typeable strains may allow for acquisition of mechanisms to further heighten virulent potential. Biofilms of NTHI, described in the middle ear of animal models as well as in children with otitis media, may confer relative resistance and impair host clearance.¹⁹ IgA proteases may play a role in NTHI virulence. Clonally related strains of NTHI expressing two IgA proteases are more frequently recovered from respiratory isolates in COPD, supporting a role for IgA proteases in the pathogenesis of infection in COPD.⁵

Host factors may also promote invasive NTHI infection. Most people with invasive NTHI infections have underlying conditions such as alcoholism, cardiopulmonary disease, HIV, cancer, splenectomy and hypogammaglobulinaemia.^{7 8} While it was tempting to ascribe this infection to our patient's immunocompromised status, we explored characteristics of his NTHI strain. The survival of NTHI from the lower respiratory tree is associated with its resistance to complement-dependent killing by human serum.³ We were intrigued that our patient's NTHI strain exhibited remarkable serum resistance and moreover that it expressed *igaA* as well as *igaB*, an additional feature of invasive NTHI strains. We were further intrigued to find that our patient's NTHI strain shared several additional features with epidemiologically unrelated NTHI strains. Notably, our strain shared identical OMP P2 sequences and nucleotide sequences, as well as SDS PAGE protein patterns, with other MLST 159 strains, including NTHI 11P6H (figure 1). While MLST 159 also includes several non-invasive strains, we made the remarkable observation that this group includes five other blood isolates of NTHI.⁵ Collectively, our studies demonstrate that our patient's NTHI strain fits a clonally related group that bears a propensity for severe NTHI infections.

We suspect this case of invasive NTHI endocarditis resulted from poor host response as well as from characteristics of the pathogen. Our investigation highlights the features of clonal strains of NTHI that have a predilection for invasive infections. While this is the first well-characterised report of NTHI endocarditis, we suspect that our patient's experience is not unique

and may have occurred in others, but may have gone unrecognised. We offer our experience to highlight special characteristics of our patient's NTHI strain, to encourage specific identification of invasive strains and to alert clinicians to the presence of invasive NTHI infections.

Learning points

- ▶ Non-typeable *Haemophilus influenzae* (NTHI), while a common cause of sinopulmonary infections, can be a cause of infective endocarditis. High-grade NTHI bacteraemia without an obvious source merits an investigation for endocarditis.
- ▶ While immunosuppression and host factors may play a role in serious NTHI infection, invasive strains, including that of our patient, bear notable common features including expression of two IgA proteases and serum resistance.
- ▶ Multilocus sequence typing confirmed that our patient's strain fits an epidemiologically unrelated clonal group of NTHI that have a predilection for invasive disease.
- ▶ We suspect that this case of NTHI endocarditis arose from a combination of impaired host response and from traits of the pathogen.
- ▶ Our experience highlights the importance of MLST 159 group of NTHI in invasive infections.

Contributors LAB-I directed patient care, wrote the majority of the manuscript, performed comprehensive review of the literature for the manuscript. TFM performed molecular analysis of patient's bacterial strain and prepared figures, wrote portions of the manuscript regarding significance of traits in comparison to other NTHI strains. TFM is a recognised authority on non-typeable *Haemophilus influenzae* pathogenesis and has made a substantial contribution to the existing literature. CK performed basic studies on bacterial virulence traits and performed molecular analyses on the bacterial strain; prepared pertinent figures. GIP directed patient's care including establishing the key diagnosis; wrote clinical portions of the manuscript. CSB responsible for writing the manuscript; responsible for manuscript integrity and for interpretation and evaluation of laboratory data, in the context of existing literature.

Competing interests None.

Patient consent Obtained.

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