

## PCR and Culture in Determination of Infectious aortic aneurysm

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### ABSTRACT

Infectious aortic aneurysm leads to poor prognosis and high mortality rate as high as 50% due to delayed/missed diagnosis. Early use of appropriate antibiotics and adequate surgical debridement would improve outcome of patients which is dependent on diagnosis on time. It takes as long as 7 days before a certain bacterium could be isolated/biochemical identified. It is difficult to differential these bacteria by the isolation/biochemical identification on time. Direct detection of a certain bacterium using molecular diagnostic assays such for PCR with specific primers is a preferred option for rapid diagnosis but requires sophisticated laboratories. A high degree of clinical suspicion should be the key for a diagnosis of infectious aortic aneurysm in endemic areas.

### INTRODUCTION

Infectious aortic aneurysm leads to poor prognosis and high mortality rate as high as 50% due to delayed/missed diagnosis. Early use of appropriate antibiotics and adequate surgical debridement would improve outcome of patients, which is dependent on diagnosis on time. It takes as long as 7 days before a certain bacterium could be isolated/biochemical identified. It is difficult to differential these bacteria by the isolation/biochemical identification on time. Even with positive result, there were a lot of bacteria with similar phenotypical, biochemical character, which also induced infective aortic aneurysm. The oftentimes implicated with infectious aortic aneurysm is *Salmonella* and *Staphylococcus*. Infectious aortic aneurysm caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) or *Burkholderia pseudomallei* (*B. pseudomallei*) has been rarely reported. The progression, roentgenographic findings and histopathology character of infected aortic aneurysm by *B.pseudomallei* is similar to those by *mycobacterium tuberculosis* (*M. tuberculosis*). A certain bacterium can be identified by PCR targeting the 16S rRNA gene, which is unambiguous for every bacterium.

### BACKGROUND

We reported a 54 years old man, who was diagnosed as infectious aortic aneurysm. Piperacillin/Sulbactam was administered. However, there was no improvement of fever and protruding lesion of the abdominal aorta on the CT image scan. Artificial vessel replacements were performed. High power view of the sections from infectious aortic aneurysm yellowish-white mass of solid consistency multinucleated Langhans giant cells, which indicates the infection of *mycobacterium tuberculosis*. But there was no evidence of tuberculosis disease. Infection with *B.pseudomallei* was suspected. Meropenem combined with oral Compound Sulfamethoxazole were given. As long as 7 days after artificial vessel replacement, the syndrome improved significantly. By the time, a gram-negative bacteria was isolated from the blood and identified as *B.pseudomallei* by phenotypical/biochemical examination and PCR targeting the 16S rRNA gene.

**KEYWORDS:** Infectious aortic aneurysm, The traditional isolation/biochemical identification, Sequence analysis of 16S rRNA gene, Melioidosis; *B.pseudomallei*, *Mycobacterium tuberculosis*, *Salmonella*, *Staphylococcus*

**ABBREVIATIONS**

*B.pseudomallei*: *Burkholderia pseudomallei*; WBC: White blood cell; NE: Neutrophile granulocyte; PPD: Tuberculin purified protein derivative; CT: Computed tomography; CRP: C-reactive protein; PCR: Polymerase Chain Reaction; 16S rRNA: 16S ribosomal RNA gene.

**MAIN TEXT**

In our study, 18 males and females with an average age of 56 years (range from 28 to 68 ages) is included. The most common responsible pathogen for infected aortic aneurysm was nontyphoid Salmonella in 12/18 patients (67%), *Staphylococcus* in 5/18 patients (27%), *B.pseudomallei* 1/18 patients (5%).

5/18 patients (27%) were treated with an endovascular stent graft. The other patients received standard treatment of performing an open repair with a resection of the infected aortic segment, extensive debridement of the surrounding tissue, and in situ or extra-anatomic bypass. During operation, gross pus was present in 1/18 patients (5%), acute suppurative inflammation was present in most cases (11/18 patients (61%)) but bacterial clumps were not commonly present (1/18 patients (5%)) [Table 1].

Presentation	symptoms	Characteristics of laboratory test	Misdiagnosis
pneumonia	fever, Cough with pyogenic sputum	Consolidation, nodular or patche in one lobe or both of lobes on CT image	Viral pneumonia; atypical pneumonia (such as Mycoplasma pneumonia)
or sepsis,	high fever	The infective marker (white blood cell (WBC), Neutrophils (NE%), C-reactive protein (CRP) were high	sepsis
localized infection	Localized pain or swelling, fever, ulceration, abscess		localized infection
Chronic infection	Fever for a long time, weight loss,	The infective marker (white blood cell (WBC), Neutrophils (NE%), C-reactive protein (CRP) were high for a long time	Tuberculosis; fever of uncertain; Typhoid fever;

**Table 1:** Characteristics, misdiagnosis and clues to diagnosis according to presentation type of melioidosis infected with *B.pseudomallei*

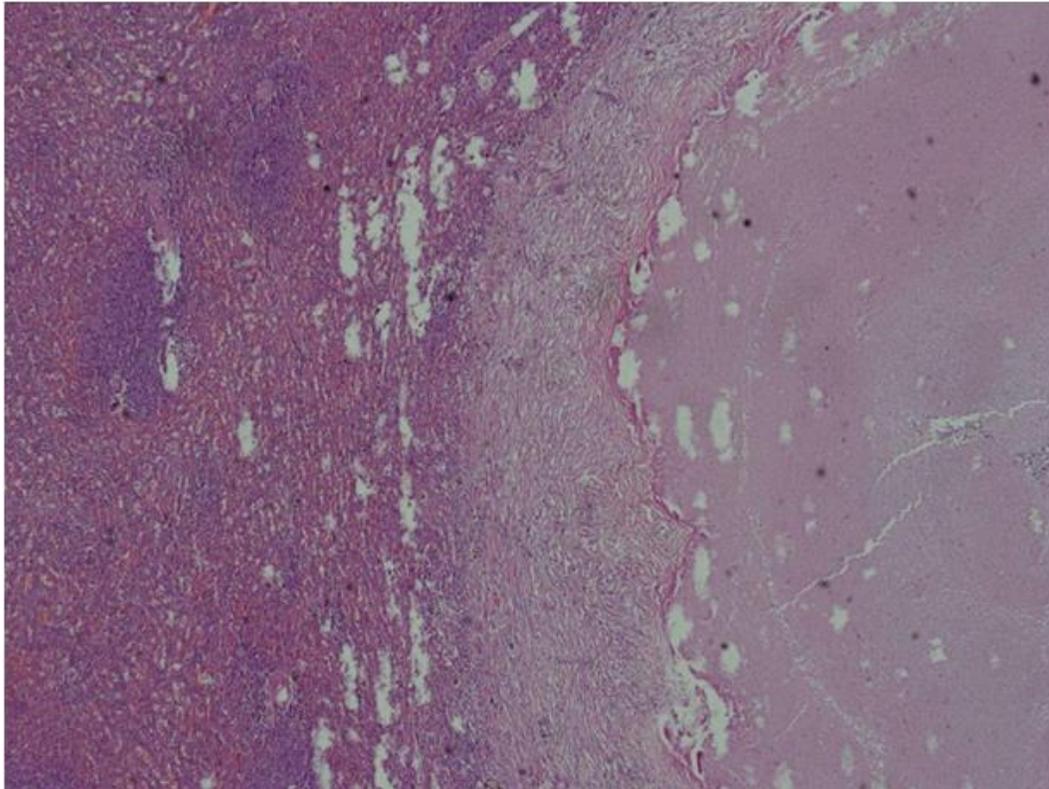
A 54-year-old man visited our hospital with gradually increasing of abdominal pain and fever for 4 months. CT showed abdominal aortic aneurysm of 2.4 × 6.0 cm with a contrast filling defect. The infective marker (white blood cell (WBC), Neutrophils (NE%), C-reactive protein (CRP) were high indicating infectious aortic aneurysm [Figure 1].



**Figure 1:** CT scans of aorta

Protruding lesion on the abdominal aortic arch with a contrast filling defect suggesting aneurysm, 2.4 × 6.0 cm.

The blood culture was taken. Piperacillin/Sulbactam was administered. The abdominal pain was remarkably relieved. However, there was no improvement of fever and protruding lesion of the abdominal aorta on the CT image scan. artificial vessel replacement was performed followed by Meropenem (0.5 every 8 hour) via intravenous injection combined with oral Compound Sulfamethoxazole (SMZco, 480mg every 12 hour) due to the risk of delayed rupture. High power view of the sections from infectious aortic aneurysm showed multiple nodules coalescing to form a large yellowish-white mass of solid consistency; multinucleated Langhans giant cells, which indicated the infection of *mycobacterium tuberculosis* [Figure 2].



**Figure 2:** High power view of sections from infectious aortic aneurysm showed a yellowish-white mass of solid consistency; multinucleated Langhans giant cells, which indicated the infection of *mycobacterium tuberculosis*.

But there was no evidence of tuberculosis disease:

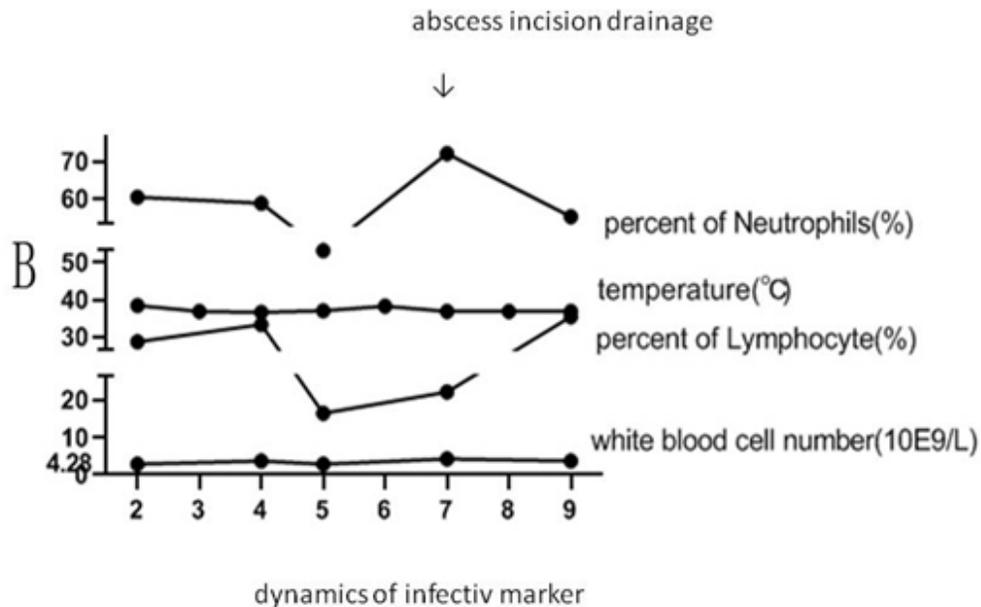
- A. The history is short
- B. It is negative for acid fast bacilli of sputum smear, tuberculin purified protein derivative (PPD) skin test.
- C. Because that tuberculosis was suspected, as well as sputum and urine sample, were negative.
- D. The responds to meropenem are very well with apparent roentgenographic improvement in 2 weeks. On the country, the treatment course for tuberculosis disease is as long as 6 months (Diagnosing treatment consist of a 4-drug regimen with rifampicin, isoniazid, ethambutol and pyrazinamide).
- E. Infectious aortic aneurysm caused by *M. tuberculosis* (tuberculous mycotic aneurysm) is often combined with miliary tuberculosis in the literature in a limited number of patients. Further study, Bronchoscopy will be given and bronchial lavage will be studied with Ziehl-Neelsen stain (*M. tuberculosis*) or hematoxylin-eosin stain (*Staphylococcus*, *Salmonella* or *B.pseudomallei*) respectively.

Further study, tissue block will be carried out by

- ① Molecular test for *M. tuberculosis*, *Staphylococcus*, *Salmonella* or *B.pseudomallei* rRNA were carried out respectively
- ② Löwenstein-Jensen culture (*M. tuberculosis*), Xylose Lysine Desoxycholate culture (*Staphylococcus*, *Salmonella*) or selective Ashdown's agar, Ashdown's selective agar (*B.pseudomallei*) of the tissue block, which will be eventually proved, confirmed diagnosis.

All above will take as long as 7 days before a certain bacterium could be isolated/biochemical identified. Even with positive result, there were a lot of bacteria with similar phenotypical, biochemical character, which also induced infective aortic aneurysm.

If 7 days after artificial vessel replacement and Meropenem combined with oral Compound Sulfamethoxazole (SMZco, 480mg every 12 hour) were given, the syndrome improved significantly. The levels of infective markers returned to normal and never increased, which indicated that the inflammation was no longer progressing [Figure 3].



**Figure 3:** Infective marker before and after abscess incision drainage

By the time, a gram-negative bacteria was isolated from the blood and identified as *B.pseudomallei* by phenotypical/biochemical examination and PCR targeting the 16S rRNA gene.

### IDENTIFICATION BY PHENOTYPICAL EXAMINATION

Preliminary identification of *B.pseudomallei* by phenotypical/biochemical examination: a Gram-negative rod with a characteristic safety pin appearance by the method of Gram bipolar staining; oxidase positive result by the method of Biochemical tests; Gentamicin and Polymyxin resistance by the method of susceptibility to antimicrobials (commercial bacterial identification systems such as bioMérieux's Analytical Profile Index 20NE and VITEK 1 & VITEK 2, which fail to distinguish *B. pseudomallei* from the closely related avirulent *B.thailandensis*).

### Identification of *B. pseudomallei* by phenotypic or genotypic identification



**Figure 4:** The morphological colonies

The next step involves definitive phenotypic or genotypic identification of *B. pseudomallei* by the method of commercial bacterial identification systems (bioMérieux's Analytical Profile Index 20NE and VITEK 1 & VITEK 2, which fail to distinguish *B. pseudomallei* from the closely related avirulent *B.thailandensis*).

### Identification of *B. pseudomallei* by sequencing of 16s rRNA

*B. pseudomallei* can be further identified by genotypic methods by the method of sequencing of 16s rRNA.

Identification of *B. pseudomallei* at the molecular level by PCR using specific *B. pseudomallei* primers continues to be a challenge due to the existing genetic similarity between closely related species of the *Burkholderia* genus.

Direct sequencing of PCR amplification products from the isolator was compatible with those of the standard *B.pseudomallei* strain K96243 (NCTC13392). Sequence analysis of the complete nucleotide/deduced amino acid sequences showed a close genetic relationship to the standard *B.pseudomallei* strain K96243 (NCTC13392), with a similarity of 99/98% for nucleotide/deduced amino acid sequence respectively. Phylogenetic analysis of the PCR amplifying product from the isolator was carried out and compared with 16S rRNA from *Salmonella*, *Staphylococcus* and *M.tuberculosis* published in GeneBank. Distance- and character-based phylogenies indicate that the isolator belongs to a species closely related to *B.pseudomallei*: using the distance-based method with the Neighbor-joining algorithm, the isolator consistently clustered with *B.pseudomallei* with strong statistical support (bootstrap over 98%). Maximum likelihood character-based phylogeny exhibited a similar topology with the isolator and *B.pseudomallei* clustering together (bootstrap over 98%). 16S rRNA amplified from the isolator and *B.pseudomallei*, *Burkholderia* spp (*B.glumae*, *B.plantarii*, *P.corrugata*, *B.glumae*, *B.caryophylli*, *B.andropogonis*, *B.vietnamiensis*, *B.cenocepacia*) were loaded from Barcode of life Date (BOLD) and added to the alignment. The thirty-seven 16S rRNA gene sequence of *B.pseudomallei* and *Burkholderia* spp were clustered into eighter groups by a neighbor-joining (NJ) tree on the value of Bootstrap 1000. The intraspecific difference in 16S rRNA gene was 0.98 in the different *B.pseudomallei* strains, 0 to 0.46 between the *B.pseudomallei* strains and *Burkholderia* spp.

Phylogenetic analysis of the PCR amplify product from the isolator by the method of neighbor-joining method (PHYLIP 3.69). Branch labels indicate bootstrap proportions in percentage of 1000 simulations. The sequence of the PCR amplifying product from the isolator and those of the reference strain published in Genbank are used.

The sequence of the PCR amplify product from the isolator indicated that the isolator is within the same genotype group of *B.pseudomallei*

We further study if the infective aortic aneurysm were induced by the infection of *B.pseudomallei*. PCR were carried out with the sample of the infective aortic aneurysm. Direct sequencing and phylogenetic analysis of PCR amplification products from the infective aortic aneurysm was compatible with that of the clinical isolator and *Burkholderia* spp. *Coreside* published in GeneBank by the neighbor-joining method (PHYLIP 3.69). Branch labels indicate bootstrap proportions in percentage of 1000 simulations. The sequence of the PCR amplify product from the infective aortic aneurysm and those of the reference strain published in Genbank are used. All were clustered into a same group, which indicated that aortic aneurysm was induced by the infection of *B.pseudomallei*.

## DISCUSSION

[1-13] Infectious aortic aneurysm is the infectious destruction of the vascular wall. Infectious aortic aneurysm is often caused by diffuse hematogenous spread of *Staphylococcus* and *Salmonella*, rarely caused by *Burkholderia pseudomallei* (*B.pseudomallei*) and *Mycobacterium tuberculosis* (*M. tuberculosis*).

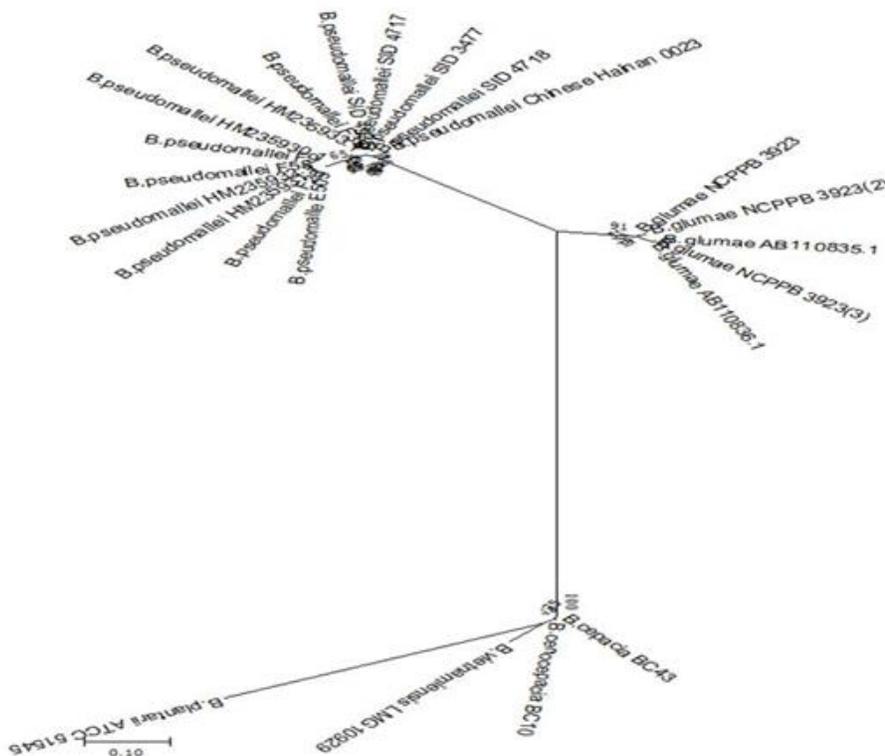
The standard treatment performed an open repair with a resection of the infected aortic segment, extensive debridement of the surrounding tissue and in situ or extra-anatomic bypass, in which there was a significant morbidity and mortality rate. Endovascular therapy (Endovascular aneurysm repair (EVAR)) provided excellent short- and medium-term results in patients with infectious aortic aneurysm and is a definite treatment or more of a "bridge-procedure" to improve a patient's condition awaiting open repair.

Melioidosis is an infectious disease caused by *B.pseudomallei*, a gram-negative intracellular bacillus. Tuberculosis, also an infectious disease, is caused by *M. tuberculosis*, an acid-fast bacillus. In both diseases, patients commonly present with fever and respiratory symptoms due to sepsis. Not only are these two entities similar in clinical presentation, but the autopsy findings may mimic each other, giving rise to difficulties in determining the cause of death. We report a case of melioidosis aortic aneurysm and compare it to a typical case of tuberculosis aortic aneurysm. Similarities between the cases on gross and histopathological examinations are discussed. In such circumstances, microbiological culture of bodily fluids and internal organs should be performed to ascertain the correct cause of death. Despite the small number of published cases to date, it is possible that the incidence of Mycotic aneurysms caused by *M. tuberculosis* maybe increase

with the increase of the number of immunodeficient patients and patients with drug-resistant tuberculosis. The other reasons consist of the infections by atypical mycobacteria and intravesical BCG injections in patients with bladder cancer. Melioidosis, which is caused by *B. pseudomallei*, has a wide range of clinical manifestations, ranging from acute fulminant sepsis to a chronic debilitating localized infection, which has been called “the great mimicker”. Melioidosis is easily misdiagnosed, making it difficult to diagnosis by clinical characteristics and radiography findings. Infectious aortic aneurysm caused by *B.pseudomallei* is seldom in Hainan province.

In both diseases infected with *B. pseudomallei* and *M. tuberculosis*, patients commonly present with fever and respiratory symptoms due to sepsis. Not only are these two entities similar in clinical presentation, but the autopsy findings may mimic each other, giving rise to difficulties in determining the cause of death. In our study, the similarities in both diseases infected with *B. pseudomallei* or *M. tuberculosis* on gross and histopathological examinations are discussed: Gross examination of the sections from the patients infected with *B. pseudomallei* showed multiple nodules coalescing to form a large yellowish-white mass of solid consistency. high power view of the sections showed multiple nodules coalescing to form a large yellowish-white mass of solid consistency; coalescing epithelioid granulomas with multinucleated Langhans giant cells (H & E, X40), which indicated the infection of mycobacterium tuberculosis. But there was no evidence of tuberculosis disease: the history is short; it is negative for acid fast bacilli of sputum smear, tuberculin purified protein derivative (PPD) skin test. Because that tuberculosis was suspected, as well as sputum and urine samples were negative. The responds to meropenem are very well with apparent roentgenographic improvement in 2 weeks. On the contry, the treatment course for tuberculosis disease is as long as 6 months (Diagnosing treatment consist of a 4-drug regimen with rifampicin, isoniazid, ethambutol, and pyrazinamide). Mycotic aneurysm is caused by diffuse hematogenous spread of *M. tuberculosis* in patient with miliary tuberculosis. Tuberculosis was active in all cases. Tuberculosis was considered because of clinical features, PPD test results, pathologic findings, and improvement on antituberculosis therapy.

As long as 7 days after artificial vessel replacement were given, the syndrome improved significantly. The levels of infective markers returned to normal and never increased, which indicated that the inflammation was no longer progressing (Figure 5). By the time, a gram-negative bacteria was isolated from the blood and identified as *B.pseudomallei* by phenotypical/biochemical examination and PCR targeting the 16S rRNA gene.



## A SUMMARY LISTING LEARNING POINTS

Infectious aortic aneurysm leads to poor prognosis and high mortality rate, which is as high as 50% due to delayed/missed diagnosis and delayed use of appropriate antibiotics/adequate surgical debridement. Laboratory confirmation of a certain bacterium leading to infectious aortic aneurysm remains a major challenge because:

It takes as long as 7 days before a certain bacterium could be isolated/biochemical identified. Even with positive result, there were a lot of bacteria with similar phenotypical, biochemical character, which also induced infective aortic aneurysm. Culture-based methods pose significant biosafety concerns of laboratory exposures.

Direct detection of a certain bacterium using molecular diagnostic assays such for PCR with specific primers is a preferred option for rapid diagnosis but requires sophisticated laboratories.

Thus, a high degree of clinical suspicion should be the key for a diagnosis of infectious aortic aneurysm in endemic areas.

## CONCLUSION

We described an attempt to identify a certain bacterium in a patient with infectious aortic aneurysm, which was confirmed by PCR and gene sequence analysis of 16S rRNA gene before a certain bacterium could be isolated/biochemical identified. It is possible to immediately identify a certain bacterium from other bacteria such as *tuberculosis bacillus*.

Ethics approval and consent to participate: The patient in the case report was available to consent. The case has been discussed with the most senior member of staff in charge of the patient's care, who has provided consent for this, and consent was obtained for use of accompanying radiological images from the consultant radiologist. The study was reviewed and approved by the Hainan Provincial People's Hospital Institutional Review Board.

## AUTHORS' CONTRIBUTIONS

Li Shi and Jie Chen participated in the design of the study, carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript; Shaohua Zhong contributed to revising the manuscript, performed the statistical analysis.

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