Trichomonads in pleural effusion: case report, literature review and utility of PCR for species identification

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SUMMARY

Trichomonas tenax is a flagellated protozoan commonly found in the human oral cavity but of unusual occurrence in pulmonary infections. We describe a case of a 67-year-old patient with glioblastoma who presented with severe pleurisy in the post-operative period while she was receiving high-dose corticotherapy. Several motile flagellated protozoa were identified in the pleural fluid. *Trichomonas tenax* was identified by molecular methods. Pulmonary infections with Trichomonads might be underestimated because of diagnostic difficulties. The utility of molecular biology for species identification is underlined and the pathogenicity of Trichomonad parasites in human lungs is discussed in light of previously reported cases.

KEY WORDS: Trichomonads, Lungs, Molecular identification, Metronidazole, Pleurisy

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INTRODUCTION

Trichomonas tenax is a flagellated protozoan commonly found in the human oral cavity, especially in patients with poor oral hygiene. Although *T. tenax* is usually considered a commensal organism, pulmonary infections caused by this parasite, which account for most of cases of pulmonary trichomonosis, have been reported repeatedly in the last few years in patients with lung disease or with various degrees of immunosuppression (Miller *et al.*, 1982; Hersh, 1985; Porcheret *et al.*, 2002; Mallat *et al.*, 2004; Wang *et al.*, 2006; Bellanger *et al.*, 2008). In most reports, identification has been made by microscopic examination that requires a high level of expertise and is often limited to the *Trichomonas* genus. Consequently, the diagnosis of pulmonary trichomonosis could clearly benefit from the use of molecular methods that allow rapid identification to the species level, as well as the discovery of new species (Mantini *et al.*, 2009).

Together with the high prevalence of Trichomonads in patients with *Pneumocystis* pneumonia and acute respiratory-distress syndrome, the question of the pathogenic power of *Trichomonas* parasites in human lungs has also been raised (Duboucher *et al.*, 2005; Duboucher *et al.*, 2007b). Herein, we report a new case of pulmonary trichomonosis, formally due to *T. tenax,* that manifested as pleurisy in a patient treated with high-dose corticotherapy for glioblastoma.

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CASE REPORT

A 67-year-old woman was admitted for left hemiparesia that had been developing for one month. Diagnosis of grade IV glioblastoma (WHO criteria) was made. Excision of a frontal lobe tumoural cyst was performed. Because of a persistent, predominantly brachio-facial, left hemiparesia in the post-operative period, high-dose corticotherapy with prednisone was started (160 mg per day, intravenously). This led to clinical improvement allowing us to plan adjuvant chemotherapy. However, on the 17th post-operative day, the patient suddenly presented with right basi-thoracic pain and acute respiratory failure that required her transfer to the intensive-care unit (ICU). She was apyretic, polypneic (up to 30/minute), and presented with severe hypoxemia (pO_2: 44 mmHg via an oxygen mask at high concentration). Antibiotherapy with amoxicilline-clavulanic acid and ofloxacin was started.

A computed chest tomography (CT) scan revealed an important right pleural effusion with pulmonary collapse (Figure 1). The pleural effusion was drained (800 mL) and was associated with respiratory improvement (decrease of oxygen dependency). The pleural fluid was sent for microbiological analysis. At macroscopic examination, the sample was of brownish appearance and purulent. Microscopic examination revealed a large number of motile and flagellated parasites, along with bacteria. Based on its microscopic characteristics, the protozoan was identified as a *Trichomonas* species (oval in shape, and ranging from 8 to 10 µm in length, with four anterior flagella and an undulating membrane). Bacterial cultures showed aero-anaerobic flora. Thus, metronidazole (1.5 g per day) was introduced and the antibiotherapy was switched to amoxicillin (6 g per day).

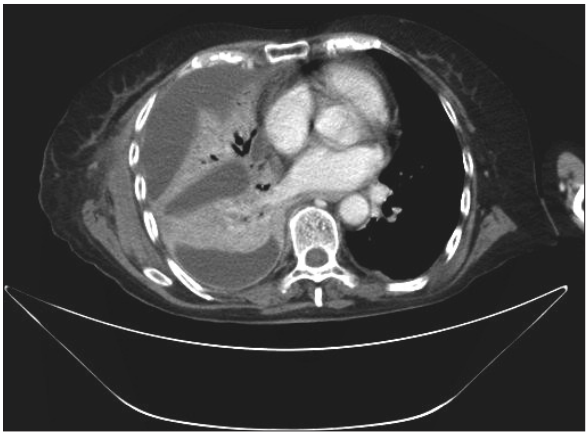

FIGURE 1 - *Chest CT scan showing the right pleural effusion.*

A sample of the pleural fluid was sent to the Laboratory of Parasitology and Mycology at Nantes University Hospital for species identification of the Trichomonads. Identification was confirmed by microscopic examination and molecular biology. Briefly, DNA was extracted from the pleural fluid using the Nucleospin Tissue kit (Macherey Nagel) according to the manufacturer's instructions. Amplification and sequencing of the 5.8S rRNA gene and internal transcribed spacer regions were achieved using the previously described primers TRICHO-F (5'-CGGTAGGTGAACCTGCCGTT-3') and TRICHO-R (5'-TGCTTCAGTTCAGCGGGTCT-3') (Jongwutiwes *et al.*, 2000). Comparisons of the nucleotide sequences of our isolate with the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) revealed a 100% homology (367 pb) with *Trichomonas tenax* ATCC 30207 (accession number U86615).

Despite the patient's initial clinical improvement and transfer out of the ICU after 6 days, by one month later our patient had died of complications of glioblastoma relapse.

The nucleotide sequence of the *T. tenax* isolate has been deposited in the GenBank database under accession number HM579936.

DISCUSSION

Pulmonary trichomonosis was initially thought to be a rare event, but studies published over the last few years indicate that its occurrence may be underestimated (Duboucher *et al.*, 2007c). Indeed, Trichomonads can present under different aspects in clinical specimens: they can occur as the typical flagellate form that is easily recognized by microbiologists, but also as an amoeboid-like form resulting from cytoskeletal changes and flagellum loss following adhesion to host cells. The latter form is particularly difficult to recognize, but is frequently reported in patients with *Pneumocystis* pneumonia and those with respiratory failure (Duboucher *et al.*, 2006).

Trichomonas tenax is often suspected to be re-

TABLE 1 - *Clinical and microbiological characteristics of the 17 cases of Trichomonads associated pleural empyema published in the literature since 1966.*

Year	*Age/sex*	*Underlying disease(s)*	*Immunosuppressive therapy*	*Microscopic examination*	*Molecular identification*	*Bacterial co-infection*	*Treatment*	*Outcome*	*References*
1966	40/M	None	no	*Trichomonas* sp.	no	no	MTZ	Clinical improvement	Abed *et al.*, 1966
1968	87/M	Chronic pulmonary disease	no	*Trichomonas tenax*	no	yes	MTZ, TET	Clinical improvement	Memik *et al.*, 1968
1973	66/M	Stomach cancer	no	*Trichomonas intestinalis* (reclassified as *Pentatrichomonas hominis*)	no	yes	MTZ	Death	Houin *et al.*, 1973
1978	35/M	Alcohol abuse	no	*Trichomonas* sp.	no	NA	MTZ	Clinical improvement	Walzer *et al.*, 1978
1982	48/M	Gastric carcinoma	no	*Trichomonas* sp.	no	yes	MTZ, GEN and CLD	Clinical improvement	Miller *et al.*, 1982
1984	NA	NA	NA	*Trichomonas* sp.	no	NA	NA	NA	Osborne *et al.*, 1984
1985	70/M	Alcohol abuse	no	*Trichomonas tenax*	no	yes	MTZ, CEF	Clinical improvement	Ohkura *et al.*, 1985
1994	42/M	Alcohol abuse	no	*Trichomonas* sp.	no	yes	MTZ	Clinical improvement	Radosavljevic *et al.*, 1994
1998	53/M	Acromegaly, rectal adenocarcinoma	Chimiotherapy, corticotherapy, cobalt irradiation	*Trichomonas tenax*	no	yes	MTZ	Clinical improvement	Shiota *et al.*, 1998
2000	28/F	Lupus erythematosus, pancytopenia	no	*Pentatrichomonas hominis*	yes	no	MTZ, AMK and CFP	Death	Jongwutiwes *et al.*, 2000
2002	59/M	Lung adenocarcinoma	Corticotherapy	*Trichomonas tenax*	no	yes	MTZ, GEN and CIP	Death	Porcheret *et al.*, 2002
2003	56/M	Diabetes mellitus type 2, subependymoma of the fourth ventricule	no	*Trichomonas* sp.	no	yes	MTZ, PTZ and TOB	Clinical improvement	Lewis *et al.*, 2003
2004	58/M	Oesophagus adenocarcinoma	no	*Trichomonas tenax*	yes	yes	MTZ, PTZ and GEN	Death	Mallat *et al.*, 2004
2006	55/M	Alcohol abuse	no	*Trichomonas* sp.	no	yes	MTZ, AMC	Clinical improvement	Wang *et al.*, 2006
2007	46/F	alcoholic liver cirrhosis	no	*Trichomonas* sp.	no	yes	MTZ, CTX	Death	Gilroy *et al.*, 2007
2008	33/F	heart transplantation	yes	*Trichomonas tenax*	yes	yes	MTZ, PTZ	Clinical improvement	Bellanger *et al.*, 2008
2009	40/F	none	no	new *Tetratrichomonas* species	yes	yes	MTZ, AMX	Clinical improvement	Mantini *et al.*, 2009

M, male; F, female; NA, not available; MTZ: Metronidazole; TET: Tetracycline; GEN: Gentamicin; CLD: Clindamycin; CEF: Cephalotin; AMK: Amikacin; CFP; Cefpirom; CIP: Ciprofloxacin; PTZ: Piperacillin-Tazobactam; TOB: Tobramycin; AMC: Amoxicillin-clavulanate; CTX: Ceftriaxone; AMX: Amoxicillin.

sponsible for the disease because of being a common commensal of the oral cavity but several other species can also be involved. As suggested by others, Trichomonad parasites probably enter the respiratory tract by aspiration of oropharyngeal secretions. Here, the portal of entry in our patient is difficult to explain, no broncho-pleural fistula being seen at CT scan. *Trichomonas* parasites are not strictly site-specific, as shown by the repeated isolation of *T. vaginalis* from respiratory-tract specimens (Carter and Whithaus, 2008). To date, at least six Trichomonads have been isolated from human lungs: *T. tenax*, *T. vaginalis, Pentatrichomonas hominis*, *Tritrichomonas foetus*, *Tetratrichomonas gallinarum*, and a newly described *Tetratrichomonas* species (Mantini *et al.*, 2009). Unfortunately, species identification within the Trichomonads is tricky and requires well-trained microscopists and fresh samples. As shown here and in previous reports, there is no doubt that diagnosis could benefit from the use of molecular tools such as ITS rDNA sequencing (Jongwutiwes *et al.*, 2000; Mallat *et al.*, 2004; Bellanger *et al.*, 2008; Mantini *et al.*, 2009).

To the best of our knowledge, the presence of Trichomonads in the pleural cavity has been reported in only 17 cases in the literature since 1966 (reviewed in Table 1). *Trichomonas tenax* accounts for most of these cases. Molecular identification has been performed only for a few of them but illustrate that distinct species could be responsible for pleural trichomonosis. As suggested by others, Trichomonad parasites probably enter the respiratory tract by aspiration of oropharyngeal secretions. In the lungs, proliferation of Trichomonads seems to depend on both the presence of bacteria, such as oral streptococci, or anaerobes and microaerophilic conditions (Radosavljevic-Asic *et al.*, 1994; Lewis *et al.*, 2003; Wang *et al.*, 2006; Bellanger *et al.*, 2008). Indeed, as shown in Table 1, bacterial co-infection, that probably allows Trichomonads to feed, is a frequent event, being noted in at least 13 of the 17 patients. All patients were given metronidazole (usually at a dosage of 1.5g IV per day) and most have a favourable outcome. The finding that all cases involving *T. tenax*, were mix infections with bacteria could suggest its moderate pathogenicity by comparison to other Trichomonads species being probably surinfecting agent. Previous studies demonstrate that some species such as *T. vaginalis* and *T. foetus* can induce apoptosis in mammalian cells but to the best of our knowledge, no experiments have been run with *T. tenax* (Singh *et al.*, 2004; Chang *et al.*, 2006). Regarding the recent literature the recovery of Trichomonads in human lungs could represent the «tip of the iceberg», Trichomonad flagellates being highly prevalent in patients with *Pneumocystis* pneumonia or acute respiratory-distress syndrome (Duboucher *et al.*, 2007a). Additionally, the zoonotic potential of Trichomonads has recently been highlighted with the description, in humans, of a flagellate close in appearance to the avian Trichomonad *T. gallinarum* (Kutisova *et al.*, 2005). The aim of this report was to draw microbiologists' attention to the potential occurrence of Trichomonad parasites in respiratory specimens and to underline the importance of direct microscopic examination of fresh, unfixed samples as well as the utility of molecular methods for species identification. It is reasonable to consider that until the question of the possible pathogenicity is clearly resolved, metronidazole should be given. Several decades after their initial discovery, Trichomonad parasites have returned to the spotlight.

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