[Original Research]

CHEST

A Pilot Feasibility Study in Establishing the Role of Ultrasound-Guided Pleural Biopsies in Pleural Infection (The AUDIO Study)

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BACKGROUND: Pleural infection is a common complication of pneumonia associated with high mortality and poor clinical outcome. Treatment of pleural infection relies on the use of broad-spectrum antibiotics because reliable pathogen identification occurs infrequently. We performed a feasibility interventional clinical study assessing the safety and significance of ultrasound (US)-guided pleural biopsy culture to increase microbiological yield. In an exploratory investigation, the 16S ribosomal RNA technique was applied to assess its utility on increasing speed and accuracy vs standard microbiological diagnosis.

METHODS: Twenty patients with clinically established pleural infection were recruited. Participants underwent a detailed US scan and US-guided pleural biopsies before chest drain insertion, alongside standard clinical management. Pleural biopsies and routine clinical samples (pleural fluid and blood) were submitted for microbiological analysis.

RESULTS: US-guided pleural biopsies were safe with no adverse events. US-guided pleural biopsies increased microbiological yield by 25% in addition to pleural fluid and blood samples. The technique provided a substantially higher microbiological yield compared with pleural fluid and blood culture samples (45% compared with 20% and 10%, respectively). The 16S ribosomal RNA technique was successfully applied to pleural biopsy samples, demonstrating high sensitivity (93%) and specificity (89.5%).

CONCLUSIONS: Our findings demonstrate the safety of US-guided pleural biopsies in patients with pleural infection and a substantial increase in microbiological diagnosis, suggesting potential niche of infection in this disease. Quantitative polymerase chain reaction primer assessment of pleural fluid and biopsy appears to have excellent sensitivity and specificity.

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KEY WORDS: bacterial infection; empyema; pleural disease; pleural infection

ABBREVIATIONS: 16S rRNA = 16S ribosomal ribonucleic acid; Ct = cycle threshold; IMRSA = *Staphylococcus aureus* methicillin-resistant; MSSA = *Staphylococcus aureus* methicillin-sensitive; qPCR = quantitative polymerase chain reaction; US = ultrasound

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Pleural infection is a common complication of pneumonia with a high mortality, affecting 80,000 patients per year in the United States and United Kingdom combined, translating to 220 new cases per day.[1] Epidemiological data from Europe and the United States suggest the incidence is increasing year on year and most of all in the elderly.[2-5] Mortality of the disease is considerable at approximately 20% in the 6 months following initial presentation.[6,7]

Treatment for pleural infection requires fluid drainage and antibiotic therapy, which is initially necessarily broad spectrum until culture results become available.[1] In up to 40% of cases of pleural infection, a microbiological diagnosis cannot be made using standard (pleural fluid and blood culture) techniques, and antibiotic treatment is empirical on the basis of local knowledge and clinical judgment.[4,5,8] This lack of a specific microbiological diagnosis leads to nonspecific and broad antibiotic treatment, potentially risking inaccurate management and contributing to poor outcomes, including the development of resistance and complications of antibiotic therapies (eg, increasing incidence of methicillin-resistant *Staphylococcus aureus*[9] and *Clostridium difficile*[10] infections).

Lack of guidance on negative blood or pleural fluid microbiology may lead to medical treatment failure, which then often means surgical intervention is required in those fit enough to undergo such management, with all the associated risks inherent to such an approach.[1,11] Methods demonstrated to increase the microbiological yield include the use of nucleic acid amplification techniques (targeting 16S ribosomal RNA sequence [16S rRNA]) that have been proposed to potentially increase overall microbiology sensitivity.[1,11]

Important questions regarding the disease microbiology remain unanswered, which may in part account for the lack of recent therapeutic advances.[12] Although infected pleural fluid is usually sampled in clinical practice because it is available for analysis, there is no direct evidence that microbes infecting the pleural preferentially inhabit the fluid. A recent animal study of pleural infection identified the presence of *Streptococcus pneumoniae* in the pleural tissue, raising questions as to whether sampling of the pleural tissue may improve diagnostics.[13] In conditions such as malignant pleural effusion and tuberculous pleuritis, it is well recognized that pleural biopsy has a much higher yield than that obtained from fluid alone.[14] It is hypothesized that, because of a rich blood supply in pleural tissue, bacteria may anchor in pleural tissue, with the minority of organisms existing in pleural fluid, but this theory has not been tested.

We hypothesized that ultrasound (US)-guided pleural biopsies would be safe and improve microbiological yield in addition to conventional methods in patients presenting with pleural infection. We aimed to assess the use of the 16S rRNA technique in combined pleural fluid and biopsy samples, using specifically designed primers for common microbes causing pleural infection.

Methods

Study Design

This was a pilot feasibility interventional study performed in two centers in the United Kingdom.

Subjects Enrolled

Study enrollment was offered to all subjects fulfilling the entry criteria at the Oxford University Hospitals NHS Foundation Trust (Oxford, UK) and Southmead Hospital (Bristol, UK). Subjects were screened during normal clinical practice and enrolled at the point of initial diagnostic pleural aspiration, which diagnosed pleural infection. Specific details about inclusion and exclusion criteria can be found in the e-Tables.

US Imaging

All patients underwent US assessment before intervention by two respiratory physicians of Royal College of Radiology Thoracic Ultrasound level I or II competence.[15] The size of effusion (small, one rib space; moderate, two to three rib spaces; large, ≥four rib spaces), echogenicity, and average number of septations per image were recorded.

Study Intervention

All patients underwent real-time US-guided pleural biopsies performed at the same procedure as chest drain insertion, using an 18-gauge Temno cutting needle with a throw of 2 cm (Temno BD).[16] The site of biopsies was determined during the US assessment by targeting the rib space with evidence of >3 cm of pleural fluid and no underlying vessels on Doppler investigation. Evidence of pleural

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