Supplemental Digital Contents

Methods

Pepsin and α-Amylase Measurements

All patients were admitted to the intensive care unit (ICU) immediately after surgery and tracheal aspirate samples were obtained within the first hour following admission (day 1), and 24 hours later (day 2). Because the specimen was collected with a suction catheter through the endotracheal tube, no particular mouthwash or decontamination protocol was required. Immediately after collection, tracheal aspirates were stored at –20°C in a freezer until they were processed. Because several patients' tracheal aspirates were very thick, all samples were first diluted (1:4) with *N*-acetylcysteine. Total (salivary and pancreatic isoenzyme) amylase and specific pancreatic amylase activities in tracheal aspirates were measured using commercially available kits (respectively alpha-amylase Ethylidene Protected Substrate and alpha-amylase Ethylidene Protected Substrate pancreatic, Roche Diagnostics, GmbH, Mannheim, Germany). Salivary amylase activity was calculated as the difference between total and pancreatic amylase activities.

Pepsin was measured quantitatively with an enzyme-linked immunosorbent assay as previously described.[1] Briefly, polystyrene flat-bottom microtiter plates (Costar CLS-3595, 96-Well, Corning, Wiesbaden, Germany) were coated overnight at room temperature with 100 µl/well of each tracheal aspirate supernatant diluted two-fold in the coating buffer (Phosphate Buffer Saline 0.1 M, pH 7.4). After washing, 100 µl of goat anti-pepsin antiserum (Interchim, Montluçon, France) diluted 1:2000 in Phosphate Buffer Saline 0.1 M, pH 7.4, were added per well and incubated for 2 hours at 37°C. After washing, 100 µl/well of conjugate solution (alkaline phosphatase-labeled rabbit anti-goat immunoglobulin G antiserum diluted at 1:2000

in Phosphate Buffer Saline 0.1 M, pH 7.4) were added and incubated for 1 hour at 37°C. Phosphatase alkaline activity was revealed by *p*-nitro-phenyl-phosphate. The pepsin concentration in each tracheal aspirate was calculated from a standard calibration curve as follows: pepsin standards (25–400 ng/ml) were prepared by serially diluting a stock porcine gastric mucosa pepsin solution (100 µg/ml) (Merck, Darmstadt, Germany) in the coating buffer. Concentrations of stock-solution standards were determined by the pepsin-extinction coefficient (millimolar extinction coefficient = 51.3 at 278 nm). A tracheal aspirate pepsin level was considered positive at a concentration of 200 ng/ml.[2]

Pepsin measurement as a marker of gastric microaspiration was well-validated in several animal and clinical studies.[3-5] Furthermore, in a randomized–controlled trial conducted on intubated critically ill patients, Nseir *et al*. clearly showed that microaspiration frequencies assessed with pepsin levels, and suspected and microbiologically documented pneumonias were significantly lower for the group with continuous cuff-pressure control.[2] Those results demonstrated that continuous cuff-pressure control, which is another intervention targeting endotracheal tube cuffs to enhance their sealing properties (like endotracheal tube cuff-shape), was associated with significantly less microaspiration of gastric contents and fewer pneumonias.

Endotracheal Cuff-Pressure Set-Up and Management

A board-certified anesthesiologist intubated enrolled patients in the operating room during general anesthesia for surgery. The type of endotracheal tube to be used was randomized prior to intubation. Based on national guidelines and standard-of-care, once the endotracheal tube was properly placed, the cuff was manually and gently inflated with air, using a 5-ml syringe. Once the endotracheal tube was connected to

the ventilator and mechanical ventilation started, cuff pressure was immediately checked and adjusted with a manual manometer to 25 cmH_2O. For both groups and from that time and throughout the study period, routine tracheal cuff care was managed by checking and adjusting cuff pressure with a manual manometer every 6 hours, following the recommendations of the two main French national critical care societies (Société de Réanimation de Langue Française, and Société Française d'Anesthésie-Réanimation).[6]

Once surgery had ended, patient was transferred to the ICU. After checking and potentially manually (with a manometer) adjusting cuff pressure, the endotracheal tube cuff was connected to the computerized cuff-pressure digital recorder for 5 hours. It is important to note that during these 5 hours, the digital recording system allowed only continuous monitoring (*i.e.*, not any pressure adjustment). Once digital recording had ended, routine tracheal cuff care was managed by checking and adjusting cuff pressure with a manual manometer every 6 hours, in accordance with the recommendations of the two main French national critical care societies (Société de Réanimation de Langue Française, and Société Française d'Anesthésie-Réanimation).[6]

Lung-Ultrasound Criteria for the Diagnosis of Ventilator-Associated Pneumonia

Lung-ultrasound patterns characterizing pneumonia have been described and validated *versus* computerized tomography-scan lung morphology in several studies.[7-9] Classically, the presence of vertical ultrasound artifacts (B lines) with irregular spacing likely show small foci of bronchopneumonia surrounded by normally aerated non-infected areas. Multiple abutting vertical B lines are representative of more severe aeration loss and extension of lung infection, whereas lung

consolidation, seen in 93% of ventilator-associated pneumonia cases, probably indicates confluent bronchopneumonia. Lung consolidations are mostly polygonal or oval with blurred margins and were found 98% of pneumonia patients' breath-dependent motion with 90% static or 8% dynamic air bronchograms. Those studies' results showed that lung ultrasound was highly accurate for pneumonia diagnosis (93% sensitivity, 98% specificity),[8] with a highly significant correlation between lung-ultrasound score and computerized tomography -scan imaging.[7,9]

Reissig *et al*.[8] estimated that the interobserver Kappa index was 0.57 (95% CI, 0.43–0.70), whereas in our ICU, Bouhemad *et al*.[7] found intra- and interobserver Kappa indexes of 0.75 and 0.70, respectively.

In our institution, every patient systematically undergoes chest x-ray and lung ultrasound at ICU admission (day 1) after major aorta surgery. Because we routinely use lung ultrasound for daily bedside assessment every time postoperative pneumonia is suspected, imaging diagnoses were made by comparing (day 1 *versus* the day of suspected pneumonia) either based on chest x-rays, when available, or lung ultrasounds acquired at those 2 times. For this study, two investigators blinded to the study-arm assignment made the final pneumonia diagnoses based on their interpretations of chest x-rays and/or lung ultrasounds.

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