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Biomarkers in the diagnosis of aspiration syndromes

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Abstract

Recognizing and managing the different types of aspiration events remain a challenging task due to the lack of distinguishing clinical or laboratory characteristics. Numerous biomarkers in serum, sputum and bronchoalveolar lavage have been studied, and their role in the recognition of aspiration remains controversial at this time. Recent animal investigations using an array of biomarkers based on distinct pathogenic features of each aspiration event have produced promising results; however, they have not been validated in humans. Newer markers are being introduced as diagnostic and prognostic tools in conditions such as community-acquired pneumonia and sepsis, but they have not been examined in aspiration. The present review summarizes the different biomarkers that have been studied in aspiration and those who might have a potential clinical use in the future.

Keywords

aspiration; aspiration pneumonia; aspiration pneumonitis; biomarkers

Aspiration is a frequently reported event in patients with abnormal level of alertness, such as patients undergoing general anesthesia [1-3], elderly and nursing home residents [4], people

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with gastrointestinal (GI) and esophageal abnormalities [5], as well as patients with neurologic and neuromuscular diseases [6]. The clinical manifestation of an aspiration event falls within a wide spectrum ranging from subclinical manifestations, such as dry cough or dysphonia to a fulminant life-threatening condition, such as the acute respiratory distress syndrome (ARDS). The pathogenic process can be further complicated by superimposed secondary infection, lung abscess, airway obstruction, exogenous lipoid pneumonia and progression into chronic interstitial fibrosis [7]. However, the most challenging task for medical practitioners is the ability to differentiate between the two most common presentations of the aspiration syndromes: the gastric aspiration pneumonitis and the bacterial aspiration pneumonia. The distinction between the two entities is important because while bacterial aspiration pneumonia needs to be treated with antibiotics, treatment of aspiration pneumonitis is supportive [8]. Routine use of antibiotics for aspiration-induced lung injury offers no benefits and promotes colonization with drug-resistant bacteria. There is overwhelming epidemiologic evidence to suggest that indiscriminate administration of antibiotics has immediate- and long-term consequences contributing to emergence of multiresistant pathogens and an increased risk of super-infection [9]. In addition, previous exposure to antibiotics is an independent risk factor for development of bacterial pneumonia in the intensive care unit (ICU) [10]. Furthermore, the use of antibiotics obscures the ability to diagnose causes of fever and infections other than in the lung. The correct identification of these problems is greatly hampered by the absence of a biomarker(s) that can truly pinpoint the diagnosis.

Numerous biomarkers have been studied in community-acquired pneumonia (CAP), nosocomial pneumonia, sepsis and other infectious diseases. Such markers have been found to guide the diagnostic and therapeutic approaches in those disorders. However, the role of these biomarkers in the management of aspiration syndromes has been inconclusive. The absence of a gold standard has been a major impediment in performing epidemiologic and therapeutic studies in this field. The majority of clinical investigations relied on clinical evidence of aspiration syndromes as their end points. These end points varied based on witnessed history of aspiration, laboratory data, oxygenation, culture of respiratory secretions and chest roentgenogram [11-13]. Other reference methods included pH monitoring [14,15], dye visualization [16], radioactive tracers [17], microbiologic analysis [18], cytology of respiratory secretions [19] and video fluoroscopic and endoscopic techniques [20]. However, none of these methods are sufficiently reliable and reproducible to be considered as a gold standard.

The purpose of this article is to review the body of information regarding existent biomarkers and discuss their potential utility in the management of aspiration events.

Pathophysiology

The pathophysiology of lung injury induced by aspiration has been studied extensively in animal preparations. Many of the studies used different aspiration insults, such as normal saline alone (NS), normal saline with adjusted acid pH (ACID), small nonacidified gastric particles (SNAP) and combined acid and small food particle (CASP). Low pH acid aspiration is a neutrophil-dependent form of lung injury that is characterized by a variable, heterogeneous injury pattern involving the bronchial airways and alveolar compartment. Type I alveolar epithelial cells undergo apoptosis or necrosis as a result of direct injury by low pH, or indirectly by release of proinflammatory mediators secondary to activation of capsaicin-sensitive (TRPV1) receptors [21,22]. This inflammatory response is contrasted to aspiration of SNAP or bacteria (or bacterial products), which directly stimulate alveolar macrophages to release innate immune/proinflammatory mediators through activation of polyanionic scavenger receptors, such as macrophage receptor with collagenous structure receptors [23] or Toll-like receptors (TLRs) that recognize different pathogen recognition products [24]. Irrespective of

the underlying aspiration insult, the lung exhibits an acute inflammatory response with patchy areas of neutrophilic inflammation, alveolar hemorrhage, intra-alveolar and interstitial edema, and impairment in the alveolar fluid clearance [25]. The damage is followed by a repair process that involves scavenging of cellular detritus by macrophages and proliferation of alveolar type II cells [26].

Aspiration of HCl occurs primarily in obstetrical patients and those with severe alcoholic intoxication [1]. Most patients aspirate complex gastric contents, which is a suspension of gastric food particulate matter, bacterial products, cytokines, and a pH that is usually higher than 1.5. Interestingly, CASP aspiration consistently results in more severe injury than acid alone or SNAP, suggesting a synergistic effect of acid and particulate matter in causing the loss of alveolar capillary border integrity [27]. In each of these settings, an increased expression of distinct biological molecules has been reported. Among these inflammatory mediators are ICAM-1/lymphocyte function-associated antigen (LFA)-1, neutrophil adhesion receptor CD18, interleukins (IL-1 β , IL-6, IL-8, IL-10), macrophage inflammatory protein (MIP)-2 and keratinocyte-derived cytokine (KC; both are rodent homologues of IL-8), monocyte chemoattractant protein (MCP)-1, TNF- α , and cytokine-induced neutrophil chemoattractant (CINC)-1. The expression profile of these cytokines varies not only with the type of aspiration but also with the time interval that elapses from the aspiration insult [28-31]. As an illustration, when TNF- α was measured in the bronchoalveolar lavage (BAL) of rats after an induced aspiration, the levels were increased. Although the expression of TNF- α did not correlate with the severity of lung injury, treatment with anti-rat TNF- α in the trachea resulted in a reduction in the lung injury in the group that was exposed to acid only, suggesting a role of TNF- α in the pathogenesis of acid-induced lung disease [28]. Moreover, the extensive influx of neutrophils during the early phase of gastric aspiration is attributed to the neutrophil chemoattractant chemokine – MIP-2. This C-X-C chemokine, which is known to induce potent chemotaxis of the neutrophils *in vitro*, is upregulated in BAL of rats' lungs after aspiration-induced lung injury. Neutralizing antibodies to MIP-2 sharply reduced neutrophil accumulation and resulted in a 33% decrease in the permeability across the alveolar capillary wall [30]. Similar findings have been demonstrated with IL-8 in rabbits following experimental acid aspiration [32]. Finally, the role of MCP-1 in acid aspiration, a chemokine that plays a role in the recruitment and activation of monocytes, was highlighted in a study done on wild-type mice and *MCP-1* gene deficient mice (*MCP-1*^{-/-}). After induced aspiration, *MCP-1*^{-/-} mice had more severe injury and worse outcome, suggesting that MCP-1 acts partly as a protector of uninjured lung zones, by promoting a process that isolates and compartmentalizes the injured areas, as observed on the lungs histopathologic exams [29].

Markers of aspiration

Pepsin—Pepsin is a digestive protease, released by the chief cells in the stomach. It derives from pepsinogen, which is its storage form in the gastric chief cells. When stimulated by gastrin and the vagus nerve, the chief cells and the parietal cells release pepsinogen and HCl respectively. The acidic pH created by HCl allows pepsinogen to undergo an autocatalytic cleavage into the active enzyme, pepsin. This acid environment is necessary for the enzymatic activity of pepsin, which is inactivated at a higher pH [33-35]. The absence of pepsin from the pulmonary and bronchial tissues makes its presence in lung aspirates an indicator of gastric content aspiration.

There are a number of investigations that have examined the role of pepsin as a biomarker for aspiration both in humans and in animal models. Using a pepsin assay developed by Anson [36] – R12 (a method in which proteolytically active pepsin is allowed to digest a known quantity of hemoglobin), Badellino *et al.* tested the above hypothesis in a rabbit model [37]. Human gastric juice (2 ml/kg) was instilled intratracheally into 24 rabbits; similar volumes of

isotonic sodium chloride solution were instilled intratracheally in control animals. BAL was performed 15, 30 or 60 min after the instillation of fluid. In the rabbits given human gastric juice, peptic activity was detected in the lavage fluid in eight out of eight animals at 15 min, six out of eight at 30 min, and five out of eight at 60 min. Because the Anson method relies on the presence of proteolytically active pepsin to digest a hemoglobin substrate, it cannot be used to detect pepsin that has been degraded in the alkaline environment of the lung. This factor explains why fewer specimens tested positive for pepsin at the 30- and 60-min times. No peptic activity was present in lavage fluid from control animals at any time. Similar findings were elicited in another experimental investigation where pepsin was detected in the tracheal aspirates of rabbits that were exposed to single or multiple gastric juice aspirations, but not in the control group [38].

Detection of pepsin in tracheal aspirate samples has been proposed as a reliable marker of gastric contents and microaspiration in children. Using, an immunoassay with rooster polyclonal antibodies to purified human pepsin, the role of pepsin as a marker of gastroesophageal reflux (GER)-related pulmonary aspiration was studied in 56 children undergoing anesthesia as part of the workup for GER. The study group was compared with positive controls, who had proven aspiration (milk suctioned from endotracheal tube), and with 13 negative controls without GER or respiratory symptoms. The positive control group had significantly higher BAL pepsin levels compared with the negative controls. However, only patients with proximal GER on pH-metry (but not distal GER) had significantly increased pepsin in BAL. Surprisingly, in children with combined distal and proximal GER, only those who complained of chronic cough had significantly increased pepsin levels [39]. Similarly, 64 children who underwent endoscopy for clinically significant GER were compared with a control group of 34 children scheduled for routine surgeries [40]. The two groups were subdivided based on the presence or absence of associated respiratory symptoms. Pepsin was detected in tracheal aspirates in seven out of eight children with history of chronic respiratory symptoms, and in 31 out of the 37 children with both reflux and chronic respiratory symptoms. It was also observed in seven out of 27 patients who had only reflux symptoms. By contrast, none of the 26 children who had neither reflux nor respiratory symptoms had pepsin in their tracheal aspirates. Overall, children with pepsin-positive tracheal aspirates were more likely to have a clinical diagnosis of GER than the pepsin-negative group [41].

In premature neonates, chronic gastric aspiration is thought to contribute to the development of bronchopulmonary dysplasia. Farhath and colleagues relied on measuring pepsin in the tracheal aspirates of neonates on ventilator support for detecting aspiration due to GER [42]. Serial tracheal aspirates obtained from 45 premature neonates revealed the presence of pepsin in 92% of the samples suggesting significant aspiration of gastric contents. In these neonates, enteral feeding was associated with a higher level of pepsin compared with unfed infants. Camacho and colleagues extended these observations by reporting the presence of pepsin in 91.4% of tracheal aspirates of 59 premature neonates of whom 31 developed bronchopulmonary dysplasia [43]. Enzymatic assays in these studies measured both pepsin and pepsinogen because secretion of pepsin is inconsistent and low in infants [44], and measuring only pepsin in tracheal aspirate samples could have underestimated the prevalence of aspiration in premature infants.

In acutely ill, mechanically ventilated adults, the validity of the pepsin assay yielded variable results. In 30 tube-fed, ventilated adults, pepsin was detectable in only 14 out of 136 (10%) tracheal aspirate samples [45]. The only variable significantly related to the detection of pepsin in these specimens was the position of the head of the bed at the time the specimens were collected. However, in a subsequent study, Metheny and coworkers demonstrated that 31.3% of 6000 tracheal secretions collected from 360 subjects tested positive for pepsin [46]. Accordingly, one or more aspiration events were noted in 88.9% of mechanically ventilated,

tube-fed participants. The percentage of pepsin-positive tracheal secretions was twice as high for patients with pneumonia on day 4 as for those without. In addition, as aspirations accumulated over the 3-day study period, the incidence of pneumonia steadily increased rising from 24% on day 1 to 48% on day 3. It was not clear what the reasons were (and none were given by the authors) behind the discrepancy in the sensitivity of the pepsin assay between the two studies.

In lung transplant patients, the suspected link between gastric aspiration and lung allograft dysfunction was studied in 13 stable lung allograft recipients. Pepsin was detected in the routine surveillance BAL of all study subjects. By contrast, it was not found in any of the four healthy controls [47]. It was noteworthy that most of the patients that were studied were being treated with a prophylactic proton pump inhibitor. Such medication would be expected to suppress symptoms associated with GER caused by acid, but a potential concern highlighted by this study is that 'clinically occult' aspiration of other gastric contents would still be possible.

There are a number of limitations that are worth mentioning regarding the use of pepsin. First, most of the human studies assumed the validity of pepsin as a marker for aspiration and did not compare it to a gold standard test. However, the relatively consistent results in animal models and human investigations suggest that pepsin might be a useful marker of aspiration, especially in patients who also have aspiration-related pulmonary diseases. Second, because pepsin activity can only be detected in the BAL for a short period of time following aspiration, only events that occur in a controlled environment where specimens can be rapidly collected (i.e., ICU) are likely to be detected. Third, a significant drawback of using molecular markers of aspiration in BAL samples is the inability to standardize the concentration. It is not known how BAL pepsin concentrations change over time following an aspiration event. Elevated levels may reflect less dilution at the time of sampling, high volume and/or high frequency aspiration events, or impaired clearance by the lung itself. Advanced molecular diagnostic techniques are currently under development for using undiluted BAL samples in detecting pepsin.

Lipid-laden macrophages—Lipid-laden alveolar macrophages (LLAMs) are macrophages in which ingested lipid can be seen inside the cells on microscopic examination. The presence of LLAMs in the BAL fluid has been considered as a marker of lipid aspiration. In 1985, Corwin and Irwin examined the validity of LLAMs as a marker of aspiration in the BAL of patients with various parenchymal lung diseases. A semiquantitative determination of a LLAM index was calculated in 49 patients with parenchymal lung diseases (nine of them were aspirators and 40 nonaspirators). Macrophages were graded by the amount of lipid in the cytoplasm of each macrophage with a score of 0–4 (0 = not opacified; 1 = up to 1/4 opacified; 2 = 1/4–1/2 opacified; 3 = 1/2–3/4 opacified; and 4 = totally opacified). A total of 100 macrophages were evaluated with a score ranging from 0 to 400. The LLAM index was increased in the lung disease group compared with controls. More importantly, LLAM indices were higher in the aspiration group than in nonaspirators. An LLAM index of 100 or greater was associated with a sensitivity and specificity of 100 and 57%, respectively. Accordingly, the authors suggested that LLAM index could be a helpful tool in excluding aspiration as a cause of parenchymal lung disease [48].

Subsequently, many studies in adults and children have reported on LLAM as marker of gastric aspiration [49–52]. The association between aspiration and LLAMs was objectively elicited when 24-h esophageal pH-monitoring was used to confirm the suspicion of GER-related aspiration in 20 children with 'difficult-to-treat' respiratory symptoms. LLAMs in BAL were higher in patients who had confirmed GER on pH-monitoring, compared with those who had negative pH-metry [53]. Similarly the 'lipid index' (reflecting the amount of lipid in 100 consecutive macrophages) was higher in the induced sputum of adults with gastric content

aspiration (defined as a fall of pH <4 at the upper esophageal electrode on 24-h pH recording), compared with those without aspiration. All 33 subjects enrolled in the study had normal chest radiographs and no clinical symptoms of aspiration [54]. These results were corroborated in children with chronic chest disease and GER compared with children with recurrent pneumonia without GER [55]. Nussbaum *et al.* compared a group of 74 children with chronic respiratory disease and documented GER to a group of 41 children with chronic respiratory disease without GER. LLAM were found in 85% of the GER group and 19% of the non-GER group ($p < 0.0001$). Published cutoff values, however, greatly varied from 67 to 200 [52,56,57] and it has been suggested that each institution must determine its own LLAM index cutoff value [58]. This recommendation was based on the fact that there was a significant overlap of LLAM indices between patient groups with underlying lung diseases. For instance, LLAM indices in children without pulmonary disease were higher than those reported for healthy adults [48]. In addition, considerable overlap of LLAM indices was observed between healthy children and those with pulmonary diseases. Only higher values yielded acceptable sensitivity and specificity for aspiration in children [59].

Recent studies did not show a significant correlation between LLAMs and aspiration. In a murine model, detection of starch granules in BAL was superior to LLAM index; the latter had a low specificity while the presence of starch granules in the BAL yielded 100% sensitivity and specificity for aspiration. The LLAM index was not different in mice that had starch aspiration versus those who had *Pseudomonas aeruginosa* instilled in their airways [60]. Similar conclusions came from human studies. While examining the correlation of LLAMs with the diagnosis of chronic pulmonary aspiration in children, Bauer and coworkers [61] found a significant overlap in the LLAM values between the aspirators and the nonaspirators group, which led the author to conclude that the LLAM index cannot stand alone as a gold standard for the diagnosis of chronic pulmonary aspiration. Furthermore, LLAM indices were found to be elevated in children with pulmonary diseases without clinical evidence of aspiration, and were similar to indices previously reported in children with pulmonary aspiration [19]. The lack of specificity of LLAMs for aspiration was reiterated when the lipid-laden index was found to be higher in children with chronic respiratory symptoms, particularly in those with cystic fibrosis, and had no correlation with the presence or absence of GER determined by intraesophageal pH monitor [52]. Other studies confirmed the lack of correlation between GER and LLAM indices, in the presence of respiratory symptoms [58,62] and in those without lung diseases [63], even when the diagnosis of GER was based on pH monitoring and endoscopy [64]. These observations suggest that increased LLAM index can be found in a variety of pulmonary disorders, and is not likely to be specific for silent aspiration. In fact, the LLAM index might be affected by other factors, regardless of the presence or absence of aspiration, as was illustrated in neonates receiving intravenous lipid infusion who had higher LLAM indices than those who were not [65]. An elevated LLAM index has also been reported in cases of pulmonary fat embolism, sickle-cell anemia, acute chest syndrome, cancer, and inhalation of organic dusts [66-68]. We would add to this list (from our personal experience) pulmonary alveolar proteinosis, chemotherapy, graft-versus-host disease and bronchiolitis obliterans.

Lipid-laden alveolar macrophages by themselves seem to lack specificity for diagnosing aspiration, even when they are assessed using quantitative methods. Their role in diagnosing aspiration and guiding its treatment is very limited at this time. Assessment of the LLAM accuracy rests on its relationship to some way of knowing whether chronic pulmonary aspiration is truly present or not. Unfortunately, a gold standard for accuracy for the diagnosis of chronic pulmonary aspiration does not exist.

Soluble triggering receptor expressed on myeloid cells 1—The triggering receptor expressed on myeloid cells (TREM), is a family of receptors expressed on polymorphonuclear neutrophils and mature monocytes, characterized by the presence of a single immunoglobulin-

like domain. It has been shown that there is an upregulation in the expression of TREM-1 after TLR activation, and soluble TREM-1 (sTREM-1) was initially thought to be a more specific marker for infection, when measured in body fluids [69-72]. However, other studies questioned the specificity of sTREM-1 for infectious processes. sTREM-1 has been shown to be increased in noninfectious disorders, such as systemic inflammatory response after multiple trauma [73] and inflammatory bowel disease [74], as well as in the synovial fluid of patients with rheumatoid arthritis [75].

The diagnostic utility of sTREM-1 in aspiration syndromes is limited by the scant investigations that are available. One study measured both blood and alveolar levels of sTREM-1 in order to differentiate between bacterial aspiration pneumonia and gastric aspiration pneumonitis. A total of 75 patients who had a witnessed aspiration event and 13 controls without underlying pulmonary disease underwent BAL and microbiological analysis of respiratory secretions. While plasma sTREM-1 levels were comparable between the controls and those with aspiration syndromes, the alveolar sTREM-1 levels were higher in the aspiration group with culture-positive BAL compared with those with culture-negative BAL. A BAL sTREM-1 cut-off value of 250 pg/ml was 65.8% sensitive and 91.9% specific for bacterial aspiration pneumonia [76]. Although promising, the usefulness of measuring sTREM-1 in clinical practice has to be determined prospectively in dedicated studies.

C-reactive protein—C-reactive protein (CRP) was first identified in 1930 from the observation of patients with pneumonia. Its name was derived from the fact that it reacted with the pneumococcal C-polysaccharide in patients' plasma during the acute phase of pneumococcal pneumonia. Subsequently, it has been recognized as a marker of inflammation, a so-called 'acute-phase protein', which is synthesized by the liver in response to infection or tissue inflammation. In response to an inflammatory insult, CRP production is rapidly stimulated by cytokines, particularly IL-6, IL-1 and TNF [77]. Although its exact function *in vivo* is not known, it probably has a role in opsonization of infectious agents and damaged cells [78,79]. Tests for CRP are now widely available and a normal circulating CRP level in a patient with symptoms of respiratory infection most likely indicates a self-limiting infection that does not require referral to hospital or antibiotic treatment.

There are limited numbers of investigations looking at the role of CRP in aspiration syndromes. The value of plasma CRP was examined in 66 comatose patients suspected of aspiration pneumonia who were admitted following drug overdose. Cultures of respiratory samples from protected BAL were used as standard for diagnosing bacterial aspiration pneumonia. While plasma CRP concentrations were increased in culture-positive and culture-negative groups, CRP levels were significantly higher in the culture-positive group. The receiver operator characteristic curve of CRP concentrations showed that a CRP greater than 75 mg/l is associated with bacterial contamination with a sensitivity of 87%, specificity of 76%, positive predictive value of 78%, and negative predictive value of 87% [80]. By contrast, two recent studies did not establish any correlation between the serum CRP and aspiration syndromes. Mylotte and colleagues [8] measured plasma CRP levels on admission and after 72 h of hospitalization in a group of nursing home patients admitted with suspected aspiration. Although the levels of CRP were increased in aspiration pneumonia, there was no statistical significance among the various groups of aspiration, including aspiration pneumonitis. Furthermore our group did not find any association between initial plasma CRP levels and severity of illness or the type of aspiration syndromes irrespective of the BAL cultures [76].

Based on the limited data available, the use of plasma CRP may not be useful in diagnosing aspiration, or in discriminating between aspiration pneumonitis and bacterial aspiration pneumonia. Whether BAL CRP in combination with other markers could have a diagnostic or prognostic value in aspiration syndromes still needs to be determined.

Procalcitonin—Procalcitonin (PCT) is a 116-amino acid peptide with a sequence identical to that of the prohormone of calcitonin [81], but PCT itself has no known hormonal activity. Under normal metabolic conditions, PCT is only present in the C cell of the thyroid gland. In bacterial infection and sepsis, intact PCT is found in the blood within 2–4 h, often reaches high values in 8–24 h (see below), and then persists as long as the inflammatory process continues (i.e., days to weeks and then persists as long as the inflammatory process continues (i.e., days to weeks). With recovery, these levels normalize. The immunoluminometric PCT assay (i.e., LUMitest, BRAHMS, Henningsdorf, Germany), which detects the ProCT prohormone and the conjoined segment of CT and calcitonin-carboxyl-peptide I is the most widely used assay to quantitate PCT. Although the sensitivity of this assay is purportedly 0.08 ng/ml (i.e., the low standard provided), the functional sensitivity is approximately 0.5 ng/ml [82].

Procalcitonin determination was first performed by Assicot *et al.* in children to differentiate bacterial from viral meningitis [83]. Since that date, PCT has become a marker of bacterial infection and there is a widening range of indications for its use. Determination of serum PCT level is now routinely performed in intensive care and surgery units in order to provide rapid evidence of bacterial origin or a shock or a respiratory distress syndrome, to differentiate pancreatitis with infected necrosis more easily from non-complicated pancreatitis, and for early detection of infectious postoperative complications [84,85]. However, PCT exhibits a lack of sensitivity in patients with focal infections [86,87]. A lack of specificity has also been reported in patients with active neoplasia, acute heart failure, myocardial infarction and recent surgery [88,89]. Other stimuli, such as smoking and inhalation burn injury, are also associated with an increased level of PCT [90].

An early study from 1996 looked at calcitonin and its precursors, including PCT, in pneumonitis. The study population consisted of 12 patients with suspected pneumonia, three suspected of aspiration pneumonitis, and 19 age-matched volunteers without lung diseases. Based on positive sputum cultures, total serum calcitonin was increased in six participants with documented bacterial infection. While PCT formed the highest fraction of total CT in the bacterial pneumonia patients, the amino-terminal PCT cleavage peptide (N-PCT) was the highest in patients who aspirated [91]. These results showed that both bacterial pneumonia and aspiration pneumonitis are associated with a rapid increase in circulating PCT and other precursor forms of calcitonin.

The utility of PCT as a diagnostic marker of gastric aspiration was studied in 23 patients who presented with closed head injury [92]. Nine patients had evidence of aspiration of gastric content, defined as the presence of persistent infiltrates on CXR in the first 24 h after admission and/or signs of aspiration on bronchoscopy. The initial serum PCT levels in these patients were significantly higher compared with those who had no evidence of aspiration. PCT was also significantly higher in nonsurvivors. It should be noted, however, that this study did not look at the role of PCT in differentiating infectious aspiration pneumonia from chemical aspiration pneumonitis.

In summary, although PCT seems to increase in aspiration and might have a prognostic value, there is currently no evidence that it helps differentiate between the different subgroups of the aspiration syndromes.

Cytokines—Multiple animal studies have demonstrated a critical role for cytokines in the pathogenesis of aspiration-related inflammatory lung injury. Many of these mediators play a role in the innate immune host response following exposure to a microbial pathogen. These regulatory peptides include the proximal proinflammatory cytokines TNF- α [28] and interleukins, IL-1 β and IL-6, the neutrophil specific chemokine IL-8 (or its rodent homolog MIP-2) [30], KC, CINC-1, and the MCP-1 [29]. Additionally, two other cytokines, IFN- γ and

IL-10, are involved in dampening the intensity of immune/inflammatory response and promoting resolution and repair.

Hutson *et al.* used proinflammatory mediators in BAL to predict the composition of gastric aspirates in a murine model [93]. Four groups of mice exposed to different type of aspirates were employed: NS, ACID, SNAP or CASP. With cytokine profile models that used a maximum of only two mediators, the four types of aspiration were correctly discriminated in 85 out of 96 rats (89%), 72 out of 78 wild-type mice (92%), and 59 out of 73 *MCP-1*^{-/-} mice (81%). The combination of acid and small gastric particles was correctly predicted in 21 out of 24 rats, 23 out of 23 wild-type mice, and 21 out of 21 *MCP-1*^{-/-} mice at 5–6 and 24 h. Predictive modeling was least accurate in terms of discriminating between the aspiration of SNAP and NS in *MCP-1*^{-/-} mice.

Depending on the aspirate type, the animal type and the time of injury, the best predictor of inflammatory mediators varied. For instance, CINC-1 at 6 h and MCP-1 at 24 h were the best predictors of CASP in rats, while TNF- α and MIP-2 at 5 h, and TNF- α and MCP-1 at 24 h were best for predicting CASP in wild-type mice (Table 1). Previous published data by the same investigators showed that the severity of aspiration-related lung injury correlated with the type of aspiration [27]. In particular, CASP was associated with more severe lung injury compared with acid or SNAP based on PaO₂/FiO₂, BAL albumin, and BAL cell number. The severity of the noninfectious gastric aspiration lung injury also correlated most strongly to levels of IL-10 in the BAL. Interestingly, unlike murine bacterial pulmonary infections, BAL IFN- γ levels were very low in all the noninfectious experimental aspiration groups [94]. Although these animal data appear promising, their application to clinical scenarios remains to be tested as aspiration events are not always as purely defined in humans as they are in the animal model. However, these results seem so far to be the most specific and accurate tools in diagnosing and discriminating the different types of aspiration syndromes. Validation of these tools in human studies might lead to a significant advancement in the management of aspiration syndromes in clinical practice.

Exhaled breath condensate leukotrienes—Leukotrienes are arachidonic acid-derived mediators that play a role in inflammation in general, and particularly in the asthmatic airways. Leukotriene B₄ (LTB₄) is a neutrophil chemotactic agent that promotes activation of these leukocytes and is thought to contribute to the accumulation of neutrophils in the lungs and airways in infectious and inflammatory diseases involving the respiratory system [95]. LTB₄ has also been shown to play a specific pathogenic role in the lung injury secondary to experimental acid aspiration [96]. In one study involving 18 children with CAP and 17 healthy controls [97], LTB₄ in exhaled breath condensate was found to be higher in children with CAP than in healthy controls. Although the technique has yet to be fully standardized, exhaled breath condensate is a promising technique for investigating inflammation in the lung, including aspiration.

Potential biomarkers—There are a number of promising biomarkers that have been useful in assessing the severity of sepsis and degree of organ injury. However, the validity of these biomarkers in clinical aspiration has not been investigated. Among these markers is carbamoyl phosphate synthase (CPS)-1. CPS-1 is a mitochondrial enzyme expressed in the hepatocytes and intestinal mucosa cells. It catalyzes a hepatic urea cycle reaction, leading to the formation of carbamoyl phosphate from ammonia and carbon dioxide. Studies examining the protein metabolism dysregulation in the liver suggested that CPS-1 release is increased in septic conditions, and data from a mouse model supported the hypothesis [98]. Whether or not CPS-1 could be useful in localized infections, such as pneumonia without sepsis, or in differentiating infectious from non infectious aspiration syndromes is still to be determined. Endothelin (ET)-1 and ET-1 precursor peptides (proET-1) are part of the endothelin system that is involved in

many pathophysiological conditions, including sepsis [99]. ET-1 levels are increased in sepsis, and higher levels of circulating ET-1 were a predictor of mortality in septic shock [100]. Furthermore, in 281 patients with CAP, proET-1 correlated with the severity of the disease and predicted mortality and ICU admission [101]. Another biomarker that has generated recent attention in animal models of infection is the receptor for advanced glycation end products (RAGE). RAGE is a multiligand receptor expressed in normal tissues and vasculature with the largest concentration in the lung [102]. Multiple studies suggested a significant role for RAGE in innate immunity and in the propagation of inflammatory response, via activation of nuclear factor (NF)- κ B and through a direct role in inflammatory cells recruitment [103]. RAGE soluble isoforms are elevated in the plasma of septic patients. In addition, higher sRAGE level was associated with worse outcome [104]. Because of the large concentration of this receptor in the lung, RAGE has also been suggested as a potential marker for type I alveolar cell injury [105]. In patients presenting with ARDS, higher baseline plasma RAGE levels correlated with the severity of lung injury and were associated with worse mortality and morbidity in the subgroup of patients ventilated with higher tidal volume (12 ml/kg) [106].

Copeptin is another 39-amino acid peptide cosynthesized with vasopressin, both derived from a larger precursor peptide [107,108]. In a prospective study of 545 patients with lower respiratory tract infections, copeptin levels correlated with the severity of the disease and higher levels were associated with worse outcome [109]. Similarly, in patients with ventilator-associated pneumonia, copeptin was an independent predictor of mortality [110]. Lastly, adrenomedullin (ADM) is a potent vasodilator 52-amino acid peptide, which in addition to its metabolic and vascular effects, plays a role in modulation of the immune response [111]. It inhibits proinflammatory cytokine production [112,113] and intracellular transduction pathways associated with apoptosis through a phosphatidylinositol 3-kinase/Akt-dependent pathway [114]. However, it is rapidly cleared from the circulation, making its measurement challenging [115]. More recently, the more stable molecule, pro-ADM, has been used as a surrogate for ADM. ProADM correlated with the severity of community-acquired pneumonia and in a receiver operating characteristic analysis for survival, the AUC for proADM was superior to PCT and CRP [116].

Expert commentary

Aspiration syndrome is a frequent problem, which particularly affects a vulnerable population and is often missed or misdiagnosed. When patients are medically evaluated after an aspiration event, it is usually for one of many possible diagnoses or pathophysiologic processes. Patients could be presenting with a reversible noninfectious aspiration-induced chemical pneumonitis, an aspiration-related bacterial pneumonia, or with ARDS. However, the classic assumption that gastric content is sterile and that bacterial pneumonia after gastric aspiration is usually a secondary superinfection following acid-induced lung injury, may not be entirely true. Aspiration of nonacidified gastric contents could clearly pick up viable endogenous flora when regurgitated into the oral pharynx before entering the trachea. Additionally, the widespread use of acid suppressive therapy, especially in the population at risk for aspiration, might change the gastric pH allowing for its colonization with bacteria [117]. Furthermore, when food particles are aspirated, it is difficult to assume that they are completely sterile, even when they are aspirated after staying in the stomach for a short duration.

Finding a way to differentiate between the distinct types of aspiration could have significant prognostic and therapeutic implications. Unfortunately, the basic pathophysiologic principles of aspiration syndromes have yet to be materialized into validated clinical tools that would provide distinct 'signatures' of aspiration pneumonitis versus bacterial aspiration pneumonia. Investigations that tested individual biomarkers in aspiration had their limitations (Table 2). Alternatively, analysis of cytokine combinations using a multiplex microarray ELISA

technique has shown promise in animal models but its relevance in clinical decision-making awaits validation in human trials. Exhaled breath condensate may provide a simple approach to biological samplings but its reproducibility remains under scrutiny. The most crucial question, however, is how to overcome the problem of a lack of a reference for the diagnosis of aspiration. Until an accurate and valid gold standard is established, the appropriate strategy is to gain additional information using different clinical and molecular techniques. The real advantage of biomarkers as an index of infection would be to unveil unrecognized facets of the clinical problem, thereby increasing the validity of clinical estimates.

Five-year view

A '5-year view' written 5 years ago would have predicted that certain biomarkers, such as sTREM-1 and PCT, would have become valid tools to differentiate aspiration pneumonia from aspiration pneumonitis, and that newer biomarkers would have been making their way to refine the clinical approach in terms of administering antimicrobial therapy, and stratifying patients into different risk categories. Unfortunately, at this time none of the available markers provide an accurate tool for guiding the diagnosis and the management of aspiration. Systems biology approaches in which microarray data are enriched with high-throughput comparative genomic and proteomic analysis may hold the key to future studies in aspiration syndromes.

Key issues

- It is difficult to differentiate the different types of aspiration syndromes on clinical grounds alone.
- Numerous biomarkers have been studied in aspiration (pepsin, lipid-laden macrophages, sTREM-1, C-reactive protein, procalcitonin and cytokines) but none has withstood the test of distinguishing the aspiration syndromes with accuracy and reliability.
- In animal models, cytokine profiling has shown promise for identifying injury-specific 'signatures' caused by gastric aspiration. Definite proof that these signatures are able to outperform the prediction of clinical parameters is still lacking.

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Table 1
Results for the prediction of type of aspiration, using inflammatory mediators in adult rats

Predicted group	Best predictive mediators
<i>Mediators at 6 h after aspiration</i>	
ACID	TNF- α , MCP-1
SNAP	MIP-2
CASP	CINC-1
<i>Mediators at 24 h after aspiration</i>	
ACID	CINC-1, MCP-1
SNAP	TNF- α
CASP	MCP-1

ACID: Normal saline plus HCl, pH = 1.25; AP: Small nonacidified particles; CASP: Combined ACID plus SNAP; CINC: Cytokine-induced neutrophil chemoattractant; MCP: Monocyte chemoattractant protein; MIP: Macrophage inflammatory protein; NS: Normal saline; SNAP: Small nonacidified gastric particles.

Table 2

Biomarkers of aspiration syndromes

Biomarker	Advantages	Limitations
Pepsin	Easy obtainable from tracheal aspirates Specific for lung aspiration	Very short window of detection Lack of standardization
Lipid-laden macrophages	Indicator of chronic lung aspiration	Low specificity Wide range of cut-off values
Soluble triggering receptor expressed on myeloid cells 1	Marker of bacterial infection	Low specificity Limited data in distinguishing between bacterial and non bacterial aspiration Lack of standardization
C-reactive protein	Marker of inflammation Rapid test available	Poor correlation between serum levels and aspiration severity Role of bronchoalveolar lavage C-reactive protein in aspiration not established
Procalcitonin	Marker of bacterial infection Quantitative test available	Limited data suggest poor discrimination between bacterial and non-bacterial aspiration Role of bronchoalveolar lavage procalcitonin in aspiration not established
Cytokines	Standardized ELISA tests Plasma- and bronchoalveolar lavage-ready Characteristic signature of aspiration pneumonitis in animal model	Not validated in human cases of aspiration