

Development and validation of a lipase nasogastric tube position test

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ABSTRACT

Background: Nasogastric tube position should be checked every day by either aspirate pH or chest radiography to prevent fatal misplaced feeding into the lungs. Many patients do not have acidic gastric aspirates and require daily chest radiographs. We developed and validated a lipase test that was compatible with non-acidic gastric aspirates.

Methods: We conducted evaluations of diagnostic test accuracy at a teaching hospital in development and validation stages. *Development:* We collected gastric and lung aspirates from 34 consecutive patients. We measured pH and human gastric lipase activity in the laboratory. These data helped us develop the lipase test. Ingenza Ltd (Roslin, Scotland) created tributyrin-coated pH test paper, which human gastric lipase converted into butyric acid, thus correcting false negatives. *Validation:* We tested nasogastric feeding tube aspirates from 36 consecutive patients with pH and lipase tests, using chest radiography or trial by use as the reference standard.

Results: *Development:* We demonstrated human gastric lipase activity in the non-acidic stomach aspirates. *Validation:* The accuracy of the lipase test (sensitivity 97.2%, specificity 100%) was significantly better than pH (sensitivity 65.7%, specificity 100%, $p < 0.05$).

Conclusions: When nasogastric tube stomach aspirates were not acidic and pH was falsely negative, the lipase test showed a true positive and was significantly more accurate.

INTRODUCTION

Nasogastric tubes are commonly used to assist enteral nutrition.¹ The National Patient Safety Agency issued guidelines recommending that aspirate pH is tested before every feed and at least once every day to check nasogastric tube position and prevent harm from feeding into the lungs through a misplaced nasogastric tube. An acidic result ($\text{pH} \leq 5.5$) indicates that the nasogastric tube is correctly positioned in the stomach and feeding is safe. If the result is not acidic ($\text{pH} > 5.5$), a chest radiograph is indicated to check that the nasogastric tube is positioned in the stomach and not in the lungs.^{2–3} No

Summary box

What is already known about this subject

- ▶ Nasogastric tube position should be checked once a day to prevent the never event of misplaced feeding into the lungs.
- ▶ Only two tests are recommended: aspirate pH and chest radiography.
- ▶ Many patients on acid-suppressing medication never have acidic gastric aspirates, which makes the pH test falsely negative.
- ▶ It is not feasible to obtain daily chest radiographs for these patients, especially in the community; therefore, there is no viable option.

What are the new findings

- ▶ We describe the development of a gastric lipase-augmented nasogastric tube aspirate pH test.
- ▶ We showed that lung aspirates have no lipase activity.
- ▶ We then showed that false-negative pH results were corrected by the lipase test, which significantly improved accuracy.

How might it impact on clinical practice in the foreseeable future?

- ▶ We intend to develop this prototype and create a more viable daily nasogastric tube position test that works for patients on acid suppression both in hospital and in the community.

tests other than pH and chest radiography are reliable and currently recommended.⁴ However, up to 42% of hospital inpatients receive antacid medications that render the results of pH test paper falsely negative.⁵

The ideal solution would be a test that was accurate despite non-acidic gastric aspirates, safe, point-of-care and non-ionising. Other authors have reported some success with pH and magnetic-tipped nasogastric tube stylets.^{6–7} The use of gastric enzymes in nasogastric tube position tests has been mooted, but no evaluations of clinically viable prototypes have been published.⁸

Human gastric lipase is an endogenous gastric enzyme, which starts the digestion of dietary triglyceride in the human stomach.⁹ Chief cells secrete human gastric lipase

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entirely from the gastric fundus.^{10 11} It is not known for certain if human lipase activity is present in the lungs. Human gastric lipase is relatively stable and its production, unlike the secretion of hydrochloric acid from gastric parietal cells, is not affected by antacid medications.^{12 13} Production of human gastric lipase is well developed at birth¹⁴ and is stimulated by pentagastrin and a high-fat diet¹⁵ and reduces with age, but does not diminish completely.¹¹ One barrier to a single reagent test is that human gastric lipase is inactivated by acidic stomach contents and therefore is unsuitable as a means of determining nasogastric tube position on its own. It has been suggested that a combined test for pH incorporating a gastric enzyme may be significantly more accurate than each in isolation.⁸ The objective of this study was to develop and validate a nasogastric tube position test that was compatible with non-acidic gastric aspirates by utilising human gastric lipase to lower the pH of gastric aspirates on pH test paper.

METHODS

Study design

We present two prospective studies. The development phase explored human gastric lipase activity from stomach and lung aspirates in the laboratory. The validation phase was a diagnostic test study that trialled the lipase test versus pH to determine nasogastric tube position reported in accordance with STARD guidelines.¹⁶

Setting

This research project was conducted in the UK at a single tertiary-referral acute London teaching hospital between 2011 and 2012. Favourable opinions were obtained from the UK National Health Service research ethics committees (Refs: 10/H0706/45 and 10/H0724/76).

Development phase

Recruitment consisted of consecutive adult patients undergoing major upper-gastrointestinal surgery involving one-lung ventilation, a procedure that ensured accurate collection of stomach and lung aspirates. Patients known to have no gastric fundus (eg, previous gastrectomy) were excluded because human gastric lipase is exclusively produced by the fundus.¹¹ All other patients who gave valid consent to participate were included. After the patient was anaesthetised, the consultant anaesthetist inserted a nasogastric tube. The consultant surgeon checked whether the tip of the nasogastric tube was correctly positioned in the stomach by palpation after gaining access to the abdominal cavity through a laparotomy incision and before mobilising any organs. This direct confirmation of nasogastric tube position represented the reference standard. After this confirmation, stomach aspirates were taken. In addition, the consultant anaesthetist took lung aspirates under direct vision by aspirating from the newly reinflated lung near the end of the operation. We immediately labelled

the samples with anonymous codes and froze them to -80°C ready for transport to the off-site laboratory. A biochemist thawed the samples and tested pH and human gastric lipase activity at the off-site laboratory. The analysis was blinded. pH was measured by wetting the pH test paper with an aspirate and waiting for 1 min (Merck, New Jersey, USA, Ref: 1095840001). Human gastric lipase activity was measured using the 718 STAT Titrino (Metrohm, Herisau, Switzerland) using methods that have already been described.¹³ A pH of ≤ 5.5 indicated correct and > 5.5 indicated incorrect nasogastric tube position. Any human gastric lipase activity indicated correct nasogastric tube position and no activity indicated incorrect nasogastric tube position.

Design of lipase test

The results of the development phase informed the creation of the lipase test. A biochemist coated pH test paper (Merck, New Jersey, USA, Ref: 1095840001) with tributyrin (Ingenza Ltd, Roslin, Scotland). This substrate produces an alcohol and butyric acid when metabolised by human gastric lipase. We hypothesised that active human gastric lipase in non-acidic nasogastric tube stomach aspirates would create enough butyric acid to change the pH on the lipase test paper to ≤ 5.5 , thus correcting false-negative results.

Validation phase

The accuracy of the lipase test was determined in the validation phase. Recruitment consisted of consecutive adult patients treated clinically with a nasogastric feeding tube. Patients known to have no gastric fundus (eg, previous gastrectomy) were excluded. All other patients who gave valid consent to participate were included. The reference standard test consisted of chest radiography or trial by use if chest radiography was not indicated. Consultant radiologists interpreted all chest radiographs while blinded to the index test results. Criteria for correct nasogastric tube position on the chest radiograph included a straight vertical course near the midline passing through the carina and not following a bronchus with the tip below the diaphragm on the same side as the gastric bubble. An aspirate from the nasogastric tube was taken within 30 min of the chest radiograph and there was no sign of nasogastric tube displacement such as change in tube length in the intervening time. If there were signs that the nasogastric tube may have been displaced, for example, patient pulling the tube, sticky plaster not securing the tube, length of tube at nares changed, then repeat aspirate tests and radiography were performed after the tube was resited. Trial by use was used because the research ethics committee deemed it inappropriate to obtain additional chest radiographs for the purpose of this study. Patients who had trial by use already had the position of their nasogastric tube satisfactorily confirmed earlier by pH, chest radiography or direct confirmation during an operation. All patients were followed up after their first

feed after entering the study had been administered and again at discharge from the hospital to ensure that no misplaced nasogastric tube feeding into the lung had occurred during their admission (ie, aspiration pneumonia). We tested the aspirate with the standard 0–6 pH test paper with 0.5 increments and also with 2–9 pH paper with 0.5 increments by wetting the test paper with aspirate (BDH, VWR International Ltd, Leicester, UK, Ref: 31505; and Merck, New Jersey, USA, Ref: 1095840001). We also simultaneously tested the aspirate with the lipase test in the same way. Two study authors (OA and MH) independently assessed the results of the test papers, while blinded to the results of the reference standard tests. The test papers were read at 1 min. A pH of ≤ 5.5 indicated correct and >5.5 indicated incorrect nasogastric tube position.

Statistics

In the development and validation phases, we compared the accuracy of pH and lipase tests in the same participants with paired analyses.¹⁷ We required at least 10 patients for the development phase.¹⁸ For the validation phase, we estimated that $n=52$ was required to rule out a clinically significant difference using pilot data (first 20 patients, pH test paper accuracy was 65%, lipase test accuracy was 100%, but 95% was used in the calculation, $\alpha=0.05$, power=80%).¹⁹ Planned interim analysis at the end of the originally allotted study time period showed a significant difference, and therefore recruitment was not extended beyond 36 patients.

RESULTS

Development phase

Participants

We recruited 36 consecutive patients who underwent upper-gastrointestinal surgery between 2011 and 2012. Two patients were not included in the analysis, because one patient withdrew consent and another patient had an inoperable tumour and the position of their nasogastric tube could not be confirmed during the operation. Therefore, data from 34 patients were included in the analysis, 23 men and 11 women. The median age of participants was 68 years (range 44–82). We obtained gastric aspirates from 32 patients (2 patients had dry gastric aspirates) and lung aspirates from 23 patients (11 patients had dry lung aspirates). Twenty-two patients (65%) were taking antacid medication (12 were taking omeprazole, 7 were taking lansoprazole and 3 were taking esomeprazole). We excluded no data from the analysis. There were no indeterminate or outlier results.

Stomach samples

The pH of the 32 stomach samples ranged from 1 to 8.5 with a mean of 4.4. 19 (59%) of the stomach samples had a pH of 5.5 or less, which would indicate correct placement of a nasogastric tube. Human gastric lipase activity was present in 21 (66%) of the stomach samples

and all of the samples between pH 3 and 8. Human gastric lipase activity was not present in samples that were more acidic than pH 3 and was also not present in a single alkaline sample at pH 8.5. This was the most alkaline sample and human gastric lipase activity was present in samples at pH 8. Crucially, 31 (97%) of the stomach samples had *either* a pH of 5.5 or less and/or human gastric lipase activity. Therefore, this indicated that a combined pH and human gastric lipase test could be more accurate than pH alone.

Lung samples

The pH of the 23 lung samples ranged from 6 to 8.5 with a mean of 6.9. None of the lung samples had a pH of 5.5 or less, which could have resulted in misplaced nasogastric tube feeding into the lung on pH criteria. Human gastric lipase activity was present in none of the lung samples, which was essential to the viability of a human gastric lipase-based test.

Validation phase

Participants

We approached 46 consecutive ward patients who were treated clinically with nasogastric tubes between 2011 and 2012. One patient could not be recruited because they had a previous gastrectomy. Nine of the recruited patients were not included in the analysis because their nasogastric tube aspirates were dry. Therefore, data from 36 patients were included in the analysis, which included 27 men and 18 women. The median age of the participants was 67 years (range 22–88). In total, 38 patients (84%) were taking antacid medication (17 were taking omeprazole, 20 were taking lansoprazole and 1 was taking esomeprazole).

Diagnostic accuracy

Table 1 shows a summary of the results. All measurements were made twice, independently, by two assessors (OA and MB) and were always in agreement with one

Table 1 Diagnostic accuracy results from the validation phase

| | | pH | + | + | – | – |
|---------------------------|-------------|----|--------|---|-----------------|---|
| Lipase test | True status | | + | – | + | – |
| + | + | | 23 | | 11 | |
| + | – | | | 0 | | 0 |
| – | + | | 0 | | 1 | |
| – | – | | | 0 | | 1 |
| | | | pH (%) | | Lipase test (%) | |
| Sensitivity | | | 65.7 | | 97.2 | |
| Specificity | | | 100 | | 100 | |
| Positive predictive value | | | 100 | | 100 | |
| Negative predictive value | | | 7.7 | | 50 | |

+, Indicates correct nasogastric tube placement; –, indicates incorrect nasogastric tube placement.

another. We excluded no data from the analysis. There were no indeterminate or outlier results. Overall, the accuracy of the lipase test was significantly greater than pH (extended McNemar's test $\chi^2=11$ with 2 df, $p<0.05$). The sensitivity of the lipase test was significantly greater than pH ($\chi^2=9.091$ with 1 df, $p<0.05$). The 95% confidence interval for the difference between the sensitivities was 17.3% to 47.5%.¹⁷

DISCUSSION

In the development study, we determined pH and human gastric lipase activity in stomach and lung aspirates. We found human gastric lipase activity in the stomach when the pH was not acidic ($\text{pH}>5.5$), which confirmed that a combined pH and lipase test might be viable and more accurate than each in isolation. We then incorporated tributyrin, a substrate to human gastric lipase, onto pH test paper. Tributyrin is metabolised by human gastric lipase to form butyric acid. This acid lowers pH and corrects false-negative results from gastric aspirates that are not acidic. We also showed that there is no human gastric lipase activity in lung aspirates, another new and crucial finding, because if there were any human gastric lipase activity in the lung, the lipase test could produce catastrophic false positives. In the second study, we examined the lipase test on aspirates from ward patients with nasogastric feeding tubes and demonstrated that it had significantly improved accuracy at determining nasogastric tube position when compared with pH.

We addressed an important unmet need in this translational research project with a novel innovative solution that can be clinically implemented and result in tangible benefits to patients, healthcare workers and organisations. We used robust methods and reported these in accordance with the STARD, QUADAS-2 and QAREL quality checklists for studies of diagnostic tests.^{16 20 21} In the validation phase, we included a spectrum of participants that is representative of the patients who will receive the test in practice. Very few patients were excluded from the study and we detailed the reasons in each case. The reference standard tests used in both studies were likely to classify the position of the nasogastric tube correctly and the index tests were performed very close to the time of the reference standard tests. The index test did not form part of the reference standard tests. No patients were lost to follow-up. In the validation phase, assessment of the index tests was independent, blinded and random with good inter-rater reliability and with the same clinical data as would be available were the test performed in practice. There were no uninterpretable, indeterminate and intermediate results or withdrawals after entering the study.

We could not include patients who were unable to give valid consent. This included some patients in the acute phases of a stroke. We did include patients with stroke who could give valid consent. Therefore, we believe that the potential spectrum bias introduced by

not including patients who cannot give valid consent does not affect the generalisability of the results. It was deemed unethical to perform additional research chest radiographs on patients who did not require them clinically by an independent research ethics committee, as trial by use as described in the methods provided an equivalent reference standard to chest radiography and more accurately represented clinical practice. The lipase test and all aspirate nasogastric tube position checks could produce a false positive result if fresh gastric contents were aspirated from the lungs. This diagnosis might be missed in patients with clinically silent aspiration pneumonia such as those in a coma. Therefore, we support the use of chest radiographs in patients at risk of silent aspiration pneumonia to check both nasogastric tube position and for radiological signs of the diagnosis.

Up to 42% of hospital inpatients receive antacid medications that render the results of pH test paper falsely negative.⁵ According to the guidelines, these patients require a chest radiograph every day. Daily chest radiography is undesirable due to radiation, cost, time and inconvenience and is inaccessible once the patient leaves the hospital. The ideal solution would be a test that was accurate despite non-acidic gastric aspirates, safe, point-of-care, intuitive and non-ionising. Other authors have reported success with pH and magnetic-tipped nasogastric tube stylets.^{6 7} These have not been widely adopted because they are operator dependent, requiring training of specialist teams that do not represent the majority of end-users. The lipase test is a viable daily nasogastric tube position check for patients in hospital and in the community. Patients with no functioning gastric fundus to secrete human gastric lipase will not benefit. The lipase test will reduce reliance on chest radiographs. A reduction in reliance on chest radiographs is desirable to minimise the delay to start feeding, exposure to radiation, cost, burden on services and misinterpretation. The National Patient Safety Agency received reports of 32 deaths and 80 severe harms associated with feeding into the lungs through misplaced nasogastric tubes between 2002 and 2010.^{2 3} Misinterpretation of chest radiographs was the most common reason for harmful misplaced nasogastric tube feeding into the lungs.^{1 22}

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