

Extra-esophageal Reflux State of the Art and Beyond in conjunction with the 7th International Symposium on Human Pepsin (ISHP), Nottingham, UK 2006

Pepsin detection for the diagnosis of Extra-oesophageal Reflux

Strugala, V.¹, McGlashan, J.A.², Morice, A.H.³, Watson, M.G.⁴, Dettmar, P.W.¹.

¹Technostics Limited, Hull UK, ²Queen's Medical Centre, Nottingham, UK, ³Castle Hill Hospital, Cottingham, UK, ⁴Doncaster Royal Infirmary, Doncaster, UK

Background:Diagnosis of extra-oesophageal reflux (EOR) follows the same well established method used for gastro-oesophageal reflux disease of ambulatory dual probe 24 hour pH monitoring (pHmetry) albeit with the upper probe being placed above the upper oesophageal sphincter in the larynx ¹. The same criteria in the oesophagus is used in the larynx with greater than 4% of a 24 hour period being spent below pH 4.0 at the upper probe being classed as abnormal. However, it is argued that any reflux event above the upper oesophageal sphincter is abnormal and therefore indicative of pathological reflux. A new diagnostic method for EOR is clearly warranted.

The contents of the stomach are a mixture of hydrochloric acid, pepsin, pancreatic enzymes and bile acids and evidence shows that the major aggressor is pepsin ^{2,3}. Since acid is neutralised by bicarbonate and food it makes more sense to use pepsin as a marker for the presence of gastric juice. A number of studies have performed pepsin testing and demonstrated the presence of refluxate in extra-oesophageal regions including larynx ^{4,5}, trachea ⁶⁻⁸, lung ⁹ and middle ear ¹⁰⁻¹².

We believe that the development of a reliable, sensitive non-invasive test for detection of reflux, that can be used as an adjunct to careful clinical examination, has the potential to become the new 'gold-standard' technique for the diagnosis of reflux disease. There will be a significant improvement in patient care and a considerable cost saving to the NHS.

Here we present data from a multi-centre pilot study evaluating the efficacy of an *in vitro* diagnostic test for reflux by detection of pepsin.

Study Protocol: The primary outcome of this study is to establish whether a newly developed non-invasive *in vitro* diagnostic test is capable of diagnosing patients with extra-oesophageal reflux disease. It is to ascertain if the novel dipstick test is able to reliably distinguish between reflux patients and normal controls by detection of the presence of pepsin in saliva.

The secondary outcome of this study is to determine the appropriate time frame in which to obtain samples from subjects. For simplicity it is easier to take a sample when the patient is attending clinic but often the patient has not had a recent reflux event and so the test will come back negative. The option that this study seeks to investigate is whether improved diagnosis can be obtained if the sample is provided at home when the patient is suffering from symptoms. An additional secondary

outcome of this study is to establish if the *in vitro* diagnostic test can be used for semi-quantification of levels of pepsin in those patients that indicate positive pepsin in their sample and to correlate data with the reflux symptom index (RSI) ¹³.

In this pilot study 20 subjects were recruited in two groups from three sites as detailed below:

- Controls n=10

Patients attending the clinic with, in the clinician's opinion, conditions distinct from extra-oesophageal reflux. They must have an RSI ≤ 3 providing the heartburn response is 0. They will be required to provide one saliva/sputum sample at the time of assessment.

- Patients n=10

Patients attending the clinic with, in the clinician's opinion, classic extra-oesophageal reflux. They must have an RSI ≥ 20 providing the heartburn response is ≥ 2 . They will be required to provide two saliva/sputum samples; one at the time of assessment and one at home when experiencing symptoms.

The saliva/sputum samples were tested for the presence of pepsin using a direct sandwich ELISA and also a lateral flow dipstick test both utilising novel monoclonal antibodies to human pepsin.

In this presentation we will present and discuss the results obtained from this pilot study.

References:

1. Postma. Otol Rhinol Laryngol 2000; 109 (10 Pt 2 Suppl 184): 10-14.
2. Johnson & Harmon. J Clin Gastroenterol 1986; 8 (Suppl 1): 26-44.
3. Tobey et al. Am J Gastroenterol 2001; 96 (11): 3062-3070.
4. Knight et al. Laryngoscope 2005; 115 (8): 1473-1478.
5. Potlurri et al. Dig Dis Sci 2003; 48 (9): 1813-1817.
6. Krishnan et al. J Pediatr Gastroenterol Nutr 2002; 35 (3): 303-308.
7. Meert et al. Pediatr Crit Care Med 2002; 3 (1): 19-22.
8. Methany et al. Am J Crit Care 2002; 11 (2): 150-154.
9. Ward et al. Thorax 2005; 60 (10): 872-874.
10. Tasker et al. Lancet 2002; 359: 493.
11. Tasker et al. Laryngoscope 2002; 112 (11): 1930-1934.
12. Lieu et al. Otolaryngol Head Neck Surg 2005; 113: 357-361.
13. Belafsky et al. J Voice 2002; 16 (2): 274-277.