Use of a non-invasive pepsin diagnostic test to detect GERD: correlation with MII-pH evaluation in a series of suspected NERD patients. A pilot study.

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Introduction

Gastroesophageal reflux disease (GERD) is one of the most common gastrointestinal disorders in Western Countries. The manifestations of GERD have been recently classified into either esophageal or extra-esophageal syndromes (EES).

Non erosive reflux disease (NERD) is a "negative" definition of a subcategory of GERD patients characterized by troublesome reflux-related symptoms in the absence of esophageal mucosal erosions without a recent acid suppressive therapy. Acid reflux remains the most important etiologic factor in the genesis of symptoms.

Pepsin is an ancient molecule and presents in all vertebrates studied, such as fishes and mammals. The peptic chief cells produce and store pepsinogen, the precursor of the active enzyme. The isoenzyme activity is at 80% of its maximum when measured at pH 1.5 and reaches its peak at pH 2. Thereafter, it declines to 45% at pH 4.5, 40% at pH 5, falls to 10% at pH 6, and ceases altogether by pH 6.5. The stability of the isoenzyme was then explored having first incubated it at 37° C for 24 hours at various pH levels, ranging from 2 to 8, and assaying at pH 3.0. The enzyme stored at pH 7.0 is inactive but stable, evidenced by the observation that ~80% of its activity is recovered when re-assayed at pH 3.0.

Recently the presence of pepsin in bronchoalveolar lavage fluid, laryngeal biopsy and sputum has been evidenced and is considered to be a consequence of GERD.

A novel non-invasive test to detect the presence of pepsin in saliva/sputum (PEP-Test) has been proposed to diagnose GERD. A correlation between PEP-Test and multichannel impedance pH monitoring (MII-pH) has never been performed.

Aim

The aim was to evaluate the accuracy of PEP-Test in diagnosing GERD using MII-pH monitoring as reference standard.

Methods

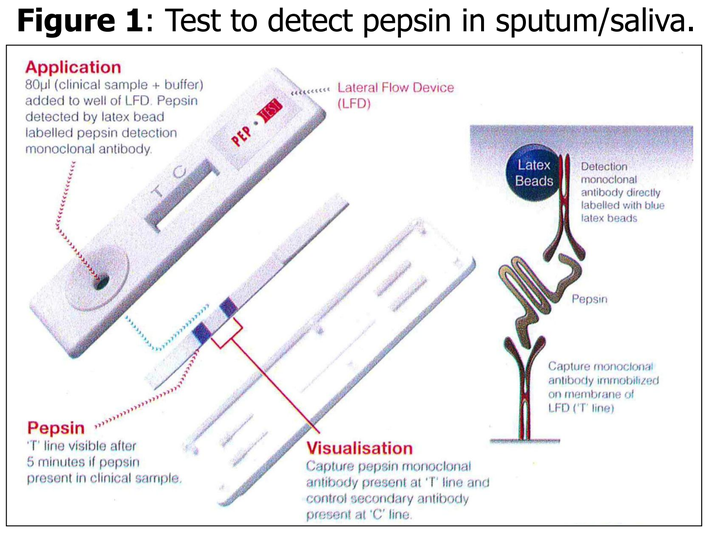
We prospectively enrolled 20 consecutive patients reporting typical (i.e. heartburn and regurgitation), atypical (i.e. belch, chest pain, dysphagia) and extra-esophageal (i.e. hoarseness, globus, laryngeal/pharyngeal pain) reflux symptoms presenting to the following outpatient units: Division of Gastroenterology, University Hospital of Genoa; Division of Gastroenterology, University Hospital of Pisa; Division of Gastroenterology, University Hospital of Padua Italy.

- **Inclusion criteria**
  - Age >18 years with informed written consent
  - Patients complaining of GERD-related symptoms since at least 6 months
  - Patients without erosive esophagitis (negative endoscopy previous PPI wash-out)
- **Exclusion criteria**
  - Patients with history of thoracic or digestive surgery
  - Patients with achalasia, scleroderma and neoplasia
  - Patients with Barrett's esophagus and/or severe erosive esophagitis (LA grade C, D)
- They were asked to discontinue PPI therapy for at least 16 days before endoscopy and motility testing

Patients underwent an initial standardized evaluation including:

a) Recording of demographics, BMI, date of diagnosis, medications, medical history including tobacco and alcohol use
b) Upper endoscopy was performed in all patients to exclude erosive esophagitis
c) Esophageal manometry was performed to evaluate esophageal motility and to detect the upper border of the lower esophageal sphincter (LES)
d) MII-pH off-therapy to detect the presence of reflux. The symptoms were considered as being related to reflux if they occurred within a 2-min time window after the onset of the reflux episode
e) Patients were categorized, by means of MII-pH results, into:
   1 True-NERD (abnormal acid exposure time (AET, %) or reflux number)
   2 Hypersensitive esophagus (HE, normal AET% and positive SAP)
   3 No-GERD patients (normal AET% and negative SAP)
f) Lateral Flow Devices (LFD) to detect pepsin was performed in all patients (Peptest™ – Euroclone)
g) Samples of saliva/sputum into a tube containing 0.01 M citric acid: within 15 minutes from experiencing reflux symptoms, and within 15 minutes after waking-up in the morning. No samples were collected after meals or intense physical activities.

Figure 1: Test to detect pepsin in sputum/saliva.



The Peptest kit is an immunological in-vitro diagnostic medical device that contains two pepsin monoclonal antibodies; it allows you to identify pepsin in a clinical sample of saliva/ sputum quickly and easily.

Levels of pepsin protein were assessed using an indirect sandwich ELISA with specific antibodies to human pepsin 3b. This method detects the presence of active and inactive pepsin.

Figure 2: Method to prepare the sample and to evaluate pepsin in sputum/saliva

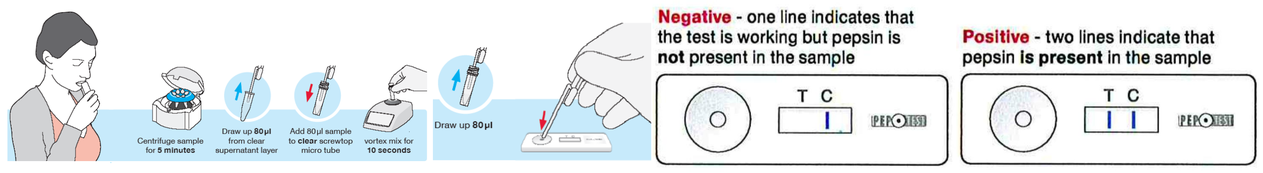
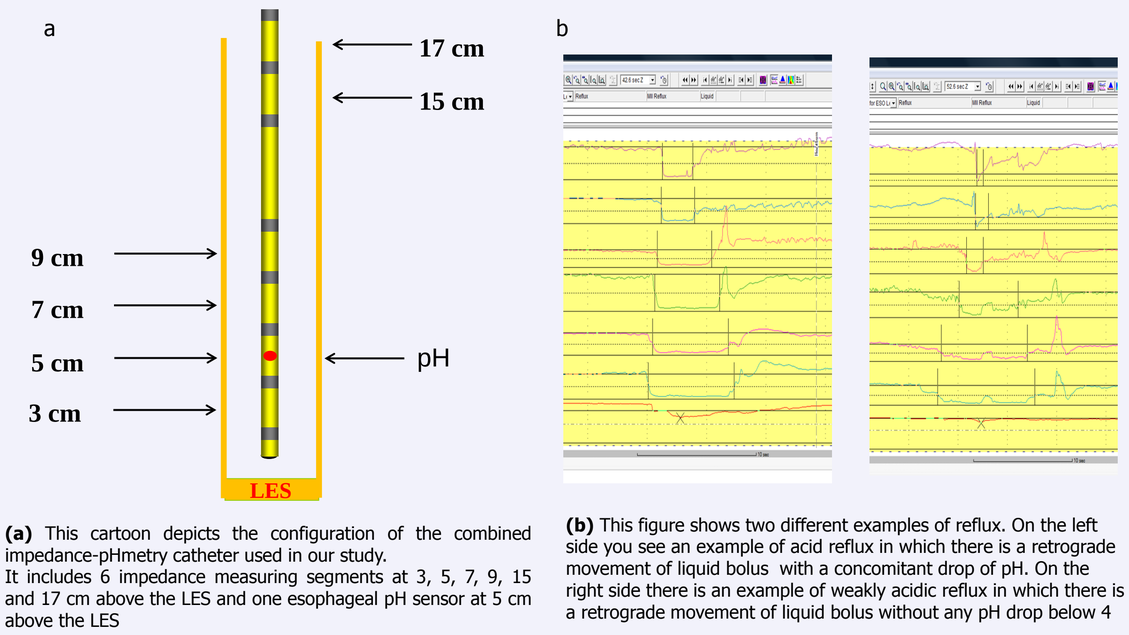


Figure 3: Combined MII – pH catheter



(a) This cartoon depicts the configuration of the combined impedance-pHmetry catheter used in our study. It includes 6 impedance measuring segments at 3, 5, 7, 9, 15 and 17 cm above the LES and one esophageal pH sensor at 5 cm above the LES

(b) This figure shows two different examples of reflux. On the left side you see an example of acid reflux in which there is a retrograde movement of liquid bolus with a concomitant drop of pH. On the right side there is an example of weakly acidic reflux in which there is a retrograde movement of liquid bolus without any pH drop below 4

Results

Male/Female was 8/12, mean age was 50.4 yrs, mean BMI was 24.8.
Six patients were regular smokers and two patients reported 2-to-3 units of alcohol consumption per day. Seven out of twenty patients presented hiatal hernia.
No patients showed abnormal esophageal motility.

MII-pH results showed: 9 True-NERD patients (median AET 10.7); 6 HE (median AET 1.6); 5 No-GERD (median AET 0.7). Peptest™ was positive in 100% of True-NERD, and in 50% of HE, and negative in 100% of No-GERD patients.

Table 1: Results of MII-pH and pepsin evaluation obtained with ELISA LFD

	M/F	Age±SD	BMI±SD	AET±SD	Prox Est (%)	SAP	Pepsine LFD positive
True-NERD	4/5	49.6±9.4	24.7±4.5	10.7±3.6	54	7/9	100%
Hypersensitive esophagus	2/4	53.3±11.5	26.1±2.7	1.6±1.2	33	6/6	50%
No-GERD	2/3	48.2±16	23.5±2.3	0.7±-1.1	32	0/5	0%

Three out of six HE patients (50%), with normal AET, normal reflux number, and positive symptomatic association (SAP), resulted positive to ELISA LFD to detect pepsin.
These patients (3) collected sputum/saliva (time recorded on a diary during 24 hours MII-pH) after a symptomatic reflux event, and the correlation with the presence of reflux evaluated with MII-pH was positive. When the association between sputum/saliva collection and reflux event evaluated with MII-pH was negative, the pepsin detection was also negative.

Table 2: Test to detect pepsin in sputum/saliva performances.

SENSITIVITY:	80%
SPECIFICITY:	100%
POSITIVE PREDICTIVE VALUE:	100%
NEGATIVE PREDICTIVE VALUE:	62.5%
ACCURACY:	85%

	Pepsin LFD positive	Pepsin LFD negative	
GERD+	12	3	15
GERD-	0	5	5
	12	8	**20**

Conclusions

Peptest™ is a simple, low-cost and safety test to detect the presence of GERD.

Overall, it demonstrates both good accuracy and high specificity in diagnosing GERD as compared to MII-pH testing.