

ORIGINAL ARTICLE

## Salivary Pepsin Test: Useful and Simple Tool for the Laryngopharyngeal Reflux Diagnosis<sup>☆</sup>



Luz Barona-Lleó,<sup>a,b,\*</sup> Claudia Duval,<sup>b</sup> Rafael Barona-de Guzmán<sup>a,b</sup>

<sup>a</sup> Clínica ORL Barona y Asociados, Hospital Casa de Salud, Valencia, Spain

<sup>b</sup> Universidad Católica de Valencia, Valencia, Spain

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### KEYWORDS

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### Abstract

**Introduction and objectives:** Laryngopharyngeal reflux (LPR) is a disease characterized by the presence of symptoms, signs and tissue damage caused by retrograde flow of gastric contents to the upper aerodigestive tract. It represents up to 10% of otolaryngology consultations.

The aim of the study is to describe the findings obtained by applying the salivary pepsin test (PEP-test) in a sample of patients with the clinical suspicion of LPR.

**Material and methods:** Our descriptive clinical study included 142 subjects with symptoms suggestive of LPR and a score above 13 on the RSI scale. The subjects underwent laryngeal endoscopy to rule out other pathologies that could justify the symptoms and the salivary pepsin test (PEP-test). The latter was carried out on fasting subjects and a second test one hour after eating, only on those with negative results.

**Results:** The results obtained in the tests performed on the 142 patients included in the study were: 105 (73.94%) presented positive results in some of the salivary pepsin tests and the results of both tests were negative in 37 subjects (26.06%).

**Conclusion:** The salivary pepsin test is a simple, low-cost, non-invasive and easily repeatable tool which could minimize empirical treatments and invasive tests for LPR diagnosis, although further research is needed for its validation.

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**Abbreviations:**  $\mu$ l, micro-litre; GERD, gastroesophageal reflux disease; LPR, laryngopharyngeal reflux; ml, millilitre; ng, nanograms; OTR, otolaryngology; RFS, Reflux Finding Score; RSI, Reflux Symptom Index; rpm, revolutions per minute; PEP-test, salivary pepsin test.

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\* Corresponding author.

E-mail address: luzbaronalleo@gmail.com (L. Barona-Lleó).

**PALABRAS CLAVE**

Reflujo  
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Saliva

## Test de pepsina en saliva: prueba útil y sencilla para el diagnóstico del reflujo faringo-laríngeo

### Resumen

**Introducción y objetivos:** El reflujo faringo-laríngeo (RFL) es una enfermedad caracterizada por la presencia de síntomas, signos y alteraciones tisulares, consecuencia del movimiento retrógrado del contenido gastrointestinal hacia el tracto aerodigestivo superior. Representa hasta el 10% de las consultas en otorrinolaringología.

El objetivo de nuestro trabajo es describir los hallazgos obtenidos al aplicar el test de determinación de pepsina en saliva (PEP-test) en una muestra de pacientes con signos clínicos sugestivos de RFL.

**Material y métodos:** En nuestro estudio clínico descriptivo se han incluido 142 sujetos con síntomas sugestivos de RFL que obtuvieron puntuaciones por encima de 13 en la escala RSI. A todos ellos se les realizó una endoscopia laríngea para descartar otras enfermedades que pudieran justificar los síntomas y el PEP-test. Ésta se realizó en ayunas a todos los sujetos, y en aquellos con resultados negativos se realizó una segunda determinación una hora después de comer.

**Resultados:** Los resultados obtenidos en las pruebas realizadas en los 142 sujetos incluidos fueron los siguientes: 105 pacientes (73,94%) presentaron resultados positivos en alguna de las determinaciones de pepsina en saliva y en 37 sujetos (26,06%) los resultados de ambas determinaciones fueron negativos.

**Conclusión:** El PEP-test es un método sencillo, económico, no invasivo y fácilmente repetible que podría minimizar el uso de tratamientos empíricos y pruebas invasivas para el diagnóstico del RFL, si bien son necesarias más investigaciones para la validación del mismo.

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## Introduction

Gastroesophageal reflux disease (GERD) is a condition that affects the digestive tract and is caused by the retrograde flow of gastric content to the oesophagus; heartburn and regurgitation are the principal symptoms. The secondary involvement of structures adjacent to the oesophagus leads to the onset of extraoesophageal manifestations.<sup>1</sup>

In 1996, Koufman proposed the term laryngopharyngeal reflux (LPR) to define the symptoms, signs and tissue abnormalities resulting from damage caused by gastric content in the upper aerodigestive tract.<sup>2</sup>

GERD with symptoms suggestive of the condition can be objectively confirmed with diagnostic tests such as pH-monitoring or high digestive endoscopy; however, the usefulness and application of these tests for LPR is controversial.<sup>3</sup> The epithelium of the upper airway is more vulnerable to injury by acid content from the stomach than the oesophageal epithelium,<sup>3,4</sup> which explains why up to 50% of patient diagnosed with LPR have no endoscopic signs to suggest oesophagitis.<sup>5</sup> Twenty-four-hour pH-monitoring is considered the gold standard for diagnosing GERD. This is a costly and invasive test that cannot be performed on all patients with clinical suspicion of LPR,<sup>6</sup> and has proven to be of low sensitivity in subjects with negative findings at digestive endoscopy.<sup>7-9</sup>

A diagnosis of LPR, in the majority of cases, is clinical and is made when there are signs and symptoms that are

“suggestive” of disease, quantified by scales such as the Reflux Symptom Index (RSI)<sup>10</sup> and the Reflux Finding Score (RFS)<sup>11</sup> and response to empirical anti-reflux treatment.<sup>12</sup> The RSI comprises 9 items, that the patient must score from 0 (normal) to 5 (severe problem); a score above 13 is “suggestive” of LPR (Table 1). The RFS comprises a set of endoscopic laryngeal signs that quantify inflammatory changes that are “suggestive” of LPR; a score above 7 is considered pathological (Table 2). Although these scales are very useful for quantifying the signs and symptoms of LPR, current medicine dictates that diagnosis and treatment must be based on confirmatory signs, symptoms and objective tests.<sup>13</sup>

The lack of specific, objective tests has raised scientific interest in finding new diagnostic tests to demonstrate and confirm LPR, and thus avoid empirical treatment.

In the references we consulted on new methods for diagnosing LPR, increasingly more authors suggest pepsin as the cause of the laryngopharyngeal signs and symptoms typical in these patients.<sup>14</sup> Pepsin is the enzyme in gastric juice that is activated by the action of hydrochloric acid from pepsinogen, and its presence in the upper aerodigestive tract, in saliva for example, can only be explained by an episode of reflux.<sup>3,15</sup>

The PEP-test is a new technology (RD Biomed Ltd., Hull, United Kingdom). It is an *in vitro* immunological method that can test for salivary pepsin simply, cheaply and noninvasively.<sup>1</sup>

**Table 1** Reflux Symptom Index.

1. Hoarseness or a problem with your voice	0	1	2	3	4	5
2. Clearing your throat	0	1	2	3	4	5
3. Excess throat mucus or postnasal drip	0	1	2	3	4	5
4. Difficulty swallowing foods, liquids or pills	0	1	2	3	4	5
5. Coughing after you eat or lying down	0	1	2	3	4	5
6. Breathing difficulty or choking episodes	0	1	2	3	4	5
7. Troublesome or annoying cough	0	1	2	3	4	5
8. Sensations of something sticking in your throat or a lump in your throat	0	1	2	3	4	5
9. Chest pain, indigestion or stomach acid coming up	0	1	2	3	4	5
Total						

Adapted from Belafsky et al.<sup>10</sup>**Table 2** Reflux Finding Score.

Subglottic oedema (pseudosulcus)	0=absent 2=present
Ventricular obliteration	0=absent 2=present 4=complete
Erythema/hyperaemia	0=absent 2=arytenoids only 4=diffuse
Vocal fold oedema	0=absent 1=mild 2=moderate 3=severe 4=polypoid
Diffuse laryngeal oedema	0=absent 1=mild 2=moderate 3=severe 4=obstruction
Posterior commissure hypertrophy	0=absent 1=mild 2=moderate 3=severe 4=obstruction
Granuloma/granulation	0=absent 2=present
Thick endolaryngeal mucus	0=absent 2=present
Total	

Adapted from Belafsky et al.<sup>11</sup>

Our study is a descriptive analysis of PEP-test findings in a sample of patients who consulted the ENT unit with symptoms suggestive of LPR. A great many of these patients with clinical suspicion of this disorder are reluctant to take anti-reflux treatment because they do not have typical GERD symptoms: heartburn and/or regurgitation.<sup>16</sup> The objective of this paper was to describe the salivary pepsin test findings in a sample of patients with clinical signs of LPR. Our aim, therefore, was to contribute towards research and the search for simple, non-invasive tests to support a clinical suspicion of LPR before starting empirical treatment or resorting to more complex diagnostic tests.

## Methods

### Subjects

The study included a sample of 142 patients, 89 females and 53 males, aged between 25 and 75 years, who attended the ENT clinic with symptoms suggestive of laryngopharyngeal reflux between February 2014 and September 2016. Subjects with RSI scores below 13 points, undergoing treatment with stomach protectors and diagnosed with infectious or tumour disease were excluded from the study.

The study sample initially comprised 187 patients; however 45 subjects were excluded during the study of the findings, because they had not complied with the established method.

### Material

All the subjects included in the sample underwent laryngeal endoscopy using a video endoscope (Henke-Sass, Wolf, GmbH) to rule out any other laryngopharyngeal disease that would have the same reported symptoms, and underwent the PEP-test (RD Biomed Ltd., Hull, United Kingdom).

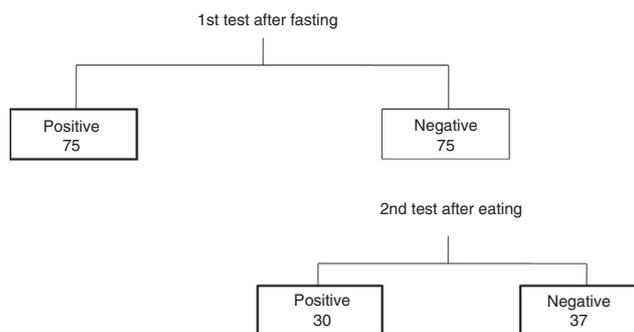
The PEP-test (RD Biomed Ltd., Hull, United Kingdom) is an immunological *in vitro* method that qualitatively tests for the presence or absence of pepsin (16 ng/ml) in a patient's saliva sample.

The patient must collect 2 ml of sputum-free saliva in a sterile 30 ml tube containing .5 ml of citric acid .01, shake it to mix the saliva with the citric acid and keep it refrigerated for up to a maximum of 7 days.

The sample is centrifuged at 4000 rpm in the laboratory for 5 min. This centrifugation creates a well-differentiated supernatant layer, from which 80 µl are extracted to be transferred to a tube containing 240 µl of migration buffer solution, and the resulting sample is mixed using a Vortex agitator for 10s. The sample is then ready to be added to the PEP-test device. Eighty µl of the sample are added to the circular device of the kit, and after a maximum of 15 min the results can be seen in the viewing window. This immunochromatographic method will show a line in the area marked C if the test has worked correctly, and if pepsin is present a line will appear in the area marked T (Fig. 1). If no C line appears, the test will be deemed void and must be repeated.



**Figure 1** Example of a positive salivary pepsin test.



**Figure 2** Salivary pepsin test results.

## Method

In our study, all the patients underwent a first salivary pepsin test after fasting, having taken no food or used any oral hygiene product.

The patients with negative results on the first test were asked to take a second sample one hour after their largest meal of the day.

A second test was not considered necessary for the subjects whose first test result was positive.

## Results

The results from the first salivary pepsin test (fasting), performed on 142 patients, were negative in 67 (47.18%), and positive in 75 patients (52.82%).

The second test results, only performed on the patients with negative results in the fasting pepsin test, were negative in 37 subjects (55.22%) and positive in 30 patients (45.78%) (Fig. 2).

The method we used did not require a second pepsin test for patients with a positive first test; nevertheless, 8 of the patients took one, which was positive in all cases.

In sum, of the 142 patients in the sample, 105 (73.94%) had positive pepsin test results, and for 37 patients (26.06%) the results of both tests were negative.

## Discussion

LPR is defined as the retrograde flow of gastric content to the upper aerodigestive tract, leading to the onset of laryngopharyngeal signs and symptoms, and accounts for, according to the series reviewed, up to 10% of ENT consultations.<sup>17,18</sup>

This paper describes the results obtained after the PEP-test for salivary pepsin in sample of patients with clinical signs suggestive of LPR, confirmed using the RSI

questionnaire. Initially the sample studied comprised 187 patients, but 45 were excluded because they did not follow the protocol correctly. For reasons that we do not know, this patient group did not take the second pepsin test required after a negative fasting PEP-test result. Therefore, eventually only 142 patients were included in the study.

After analysing the data obtained from the 142 patients included in the sample our results show that 76.94% of patients had a positive result for one of the tests, whereas 26.06 had negative results for both tests.

In routine clinical practice, generally, a diagnosis of LPR is made with the help of the RSI and RFS questionnaires. The RSI is a subjective test to be completed by the patient, and authors such as Hicks et al., Milstein et al. and Spyridoulas et al. agree that its specificity is low, since symptoms such as cough, throat clearing, dysphonia, etc., can occur in other diseases that involve laryngopharyngeal irritation or inflammation.<sup>19-21</sup> The RFS covers endoscopic signs of laryngeal inflammation; scores above 7 are suggestive of LPR. Some of the authors consulted consider it an imprecise method, since these signs can appear in other laryngeal diseases, and therefore misdiagnosis is possible.<sup>22,23</sup> Belafsky et al. showed the intra and interobserver reproducibility of the RFS when used by specialist laryngologists<sup>11</sup>; however, most of these patients are attended by general otolaryngologists. Chang et al. performed a study analysing the interobserver reproducibility of the RFS when used by non-specialist otolaryngologists and concluded that in these cases the results of the RFS did not coincide between the different examiners.<sup>22</sup>

Our results show that negative results were obtained by 26.06% of the patients in both tests. This data supports that of other authors who value the RSI and RFS questionnaires as useful, but who acknowledge their limitations, *i.e.*, the low specificity of the former and the poor reproducibility of the latter. Therefore, although LPR was clinically suspected in our patients, we expected that this diagnosis would not be confirmed for all of them and that their clinical signs would have a different cause.

A favourable response to empirical anti-reflux treatment is another method used by some researchers to confirm a diagnosis of LPR.<sup>12</sup> However, Qadeer et al. published a meta-analysis that summarized randomized controlled trials on PPI prescribed for patients with suspected LPR, and concluded that there were no statistically significant differences between the PPI and the placebo.<sup>24</sup> The financial cost and the adverse effects that can occur after empirical anti-reflux treatment also motivate the search for new diagnostic methods.<sup>9</sup> The 2013 American gastroenterological guideline argues against empirical treatment if there are endoscopic laryngeal signs with no heartburn or acid regurgitation.<sup>25</sup>

Friedman et al., in a study undertaken and published in 2012, showed that a diagnosis of LPR cannot be made on the basis of symptoms alone, and concluded that diagnosis and treatment should be based on a combination of symptoms, signs and confirmatory testing.<sup>13</sup> There is currently no gold standard test for diagnosing this disorder. Twenty-four-hour pH-monitoring and upper digestive endoscopy are the tests of choice to confirm a diagnosis of GERD; however, these are low sensitivity tests for LPR.<sup>7-9</sup>

In recent years, the high prevalence of LPR, its financial cost and the lack of a gold standard test for its diagnosis,

have encouraged researchers to search for new simple and low-cost tests to improve the quality of life and management of these patients at a lower cost.

Spyridoulas et al. suggested in their study that the presence of pepsin in the upper airway could be a potential biomarker to objectify LPR, based on the fact that pepsin is an enzyme in gastric juice that is activated in the presence of hydrochloric acid and might indicate reflux if found in saliva.<sup>19</sup> The PEP-test was recently developed to test for this (RD Biomed Ltd., Hull, United Kingdom). The test uses 2 unique monoclonal antibodies against human pepsin 3; one to detect and the other to capture pepsin if present in a saliva sample.<sup>1,26</sup> Since this is a novel device and a recent diagnostic technique, there are few studies on it in the literature consulted. However, in recent years various authors have shown an interest in demonstrating the usefulness of determining salivary pepsin as a potential confirmatory test for LPR.

Sarita et al., and subsequently Hayat et al., performed studies comparing the results obtained from salivary pepsin testing in 2 groups of patients: a control group and a group with typical GERD symptoms (heartburn and regurgitation). Both coincided in that the patients with symptoms suggestive of reflux had a greater prevalence and concentration of salivary pepsin than the controls. Therefore, they suggest that this test should complement the RSI and RFS questionnaires, and thus reduce unnecessary anti-reflux treatment and more costly invasive tests.<sup>1,9</sup>

Wang et al. published a study correlating high scores in the RSI and RFS questionnaires with high salivary pepsin levels.<sup>27</sup>

Recently, Na et al., who coincide with other authors on the usefulness of salivary pepsin testing in diagnosing LPR, published a study aimed at clarifying the optimal timing for the salivary pepsin test. After studying a sample of 69 patients (12 controls and 57 with symptoms suggestive of LPR) they concluded that it should be performed on waking.<sup>6</sup> Using this article as our benchmark, for financial reasons, a second test was not requested for the 75 patients with a positive result in the first PEP-test. However, 8 of these 75 patients did undergo a second test voluntarily for reasons that we do not know. The coincidence between both results in all of the cases supports the method we used in our study as appropriate and that if the fasting pepsin test is positive, it is not essential to perform a second test.

The sensitivity and specificity of salivary pepsin testing are not perfect and have limitations for diagnosing LPR, like other techniques such as pH-monitoring or digestive endoscopy. However, the test does have several advantages over these techniques in that it is low-cost (approximately 30 Euros in our environment), non-invasive, easy-to-interpret and repeatable.<sup>9</sup>

It is obvious that the presence of a substance, in this case pepsin, in a location other than the usual must be due to an anomaly. Even so, this is a very new area that requires further research studies to clarify certain aspects that are generating debate. Dhillon et al., in one of their publications, explain that physiological reflux exists and that although testing for salivary pepsin might indeed be interesting to objectify LPR, further research is necessary on the subject.<sup>3</sup> Nonetheless, we must stress that in this study, all the patients in the sample had symptoms that were

suggestive of LPR with scores above 13 on the RSI scale. Future research studies should also focus on any changes before and after administering anti-reflux treatment.<sup>28</sup>

## Conclusion

In sum, LPR presents very commonly in ENT consultations and we currently have no gold standard test to confirm clinical suspicion based on laryngopharyngeal signs and symptoms.

From this descriptive study we consider that the PEP-test is simple, low-cost, non-invasive, repeatable and easy-to-interpret and could be used as a complementary test to support and confirm a clinical suspicion of LPR based on the RSI and RFS questionnaires. Similarly, it would help towards limiting the use of empirical treatment and more complex and costly invasive tests, which would be reserved for specific cases.

However, although we consider that this test might be very useful in routine clinical practice, it is clear that further research studies are required that compare the results from this test with other exploration methods used to detect LPR, and to assess changes in salivary pepsin tests before and after medical treatment.

## Conflict of Interests

The authors have no conflict of interests to declare.

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