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# High Resolution Manometry to detect Transient Lower Esophageal Sphincter Relaxations: diagnostic accuracy compared to perfused-sleeve manometry and definition of new detection criteria.

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10 **Short title** : High resolution manometry to detect tLESRs  
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13 **Keywords** : High resolution manometry, transient lower esophageal sphincter relaxation,  
14 healthy volunteers  
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19 **Abbreviations:** high resolution manometry (HRM), lower esophageal sphincter (LES),  
20 esophageal pressure topography (EPT), perfused-sleeve manometry (PSM), transient lower  
21 esophageal sphincter relaxation (tLESR), upper esophageal sphincter (UES)  
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## Summary

**Background.** Inhibition of transient lower esophageal sphincter relaxations (tLESRs) has become one of the most relevant therapeutic objective in patients with reflux symptoms resistant to proton pump inhibitors. TLESRs are currently detected by esophageal perfused-sleeve manometry (PSM), but esophageal high resolution manometry (HRM), which combines closely spaced pressure sensors and esophageal pressure topography plots, may prove to be a better tool.

**Aim.** The aim of this study was to evaluate the efficacy, reproducibility and inter-observer agreement of HRM for the detection of tLESRs, in comparison with PSM.

**Methods:** Twenty-four healthy volunteers underwent HRM alone and on a separate occasion with PSM simultaneously. LES pressure was monitored for 1 hour during fasting and 2 hours postprandial. Criteria for tLESRs were defined by characterizing spontaneous LES relaxation associated with common cavity, and then applied to all spontaneous LES relaxations. Inter observer agreement and the rates of tLESRs detected by HRM and PSM were compared.

**Results:** New HRM criteria for the detection of tLESRs have been established. A similar number of tLESRs were identified during the 2 HRM recordings (median per subject 15 and 13 ( $p=0.07$ ), and less with PSM (median per subject 11,  $p<0.01$ ). The overall concordance rate between the 2 procedures was substantial ( $\kappa=0.61$ ). The inter-observer agreement was almost perfect ( $\kappa=0.83$ ) with HRM and only fair ( $\kappa=0.38$ ) with PSM.

**Conclusions:** HRM is reproducible and more sensitive than PSM to detect tLESRs. HRM provides a better inter-observer agreement. These results confirm that HRM is the gold standard for detecting tLESRs (NTC00931593).

**Abstract word count:** 248

## Introduction

Gastro-esophageal reflux disease (GERD) is primarily a motility disorder in which impaired lower esophageal sphincter (LES) function plays a crucial role. Thirty years ago, it was established that most reflux episodes result from transient relaxations of the LES (tLESRs) rather than from low resting LES pressure alone [1, 2]. More recently, studies using impedance-pH monitoring have demonstrated that 30-40% of GERD patients have persistent symptoms despite proton pump inhibitor therapy related to non-acid reflux events [3, 4]. Since tLESRs represent the main mechanism of all types of reflux events [5], controlling the occurrence of tLESRs is considered to be a relevant therapeutic objective in GERD and has induced intense pharmacological research to develop anti-reflux therapies [6].

TLESRs are characterized by a complete and long lasting decrease of the lower esophageal sphincter pressure not preceded by swallowing and usually accompanied by inhibition of the crural diaphragm [7]. Using perfused-sleeve manometry (PSM), Holloway et al. defined objective criteria for tLESRs [8]. These criteria take into account the absence of swallow, the rate of relaxation, the nadir LES pressure and the duration of LES relaxation. Inhibition of crural diaphragm and prominent after contraction have been recently added to the original criteria to reduce intra and inter observer variability [9]. However PSM has some limitations. It exhibits slow transient response to pressure variations and examination in supine position is preferable to minimize the hydrostatic effects on pressure measurement. Moreover the review of tracings is difficult and consensus analysis is mandatory due to inter observer variability [9].

High resolution manometry (HRM) offers several advantages over standard manometry. The catheter has more recordings sites and less space between them, allowing the complete definition of intraluminal pressure and the reduction of movement-related artifacts. A seamless and dynamic representation of pressure variations is then available from the upper esophageal sphincter (UES) to the esophago-gastric junction. Moreover the

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6 pressure variations are displayed as esophageal pressure topography plots (EPT) and this  
7 representation might facilitate data interpretation [10]. Therefore, HRM might improve the  
8 detection of tLESRs.  
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11 HRM has already been used in different studies to detect tLESRs. Holloway's criteria  
12 initially designed for PSM were applied to HRM to identify tLESRs [11-16]. Bredenoord et al.  
13 compared the sleeve sensor and HRM with water-perfused sensors for the detection of  
14 tLESRs in healthy volunteers [12]. It was concluded that HRM was at least as accurate as  
15 sleeve sensors. However this study was not a direct comparison of the 2 techniques: the  
16 authors used the same manometric catheter to collect data and performed 2 analyses, one  
17 using the classic sleeve sensor mode tracings and one using the isocontour HRM mode.  
18 Other studies using HRM with solid-state pressure sensors have been performed but cannot  
19 be considered as validation studies since no definition of tLESR in HRM was proposed and  
20 no direct comparison with PSM was performed [11, 13-16].  
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34 Therefore, our aims were to define objective criteria for tLESRs detection in HRM with  
35 solid-state pressure sensors and to compare directly the diagnostic accuracy of PSM and  
36 HRM for detecting the occurrence of tLESRs.  
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## 43 **Methods**

### 44 **Subjects and study protocol**

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47 Twenty-eight healthy volunteers were recruited to participate in this study. None of  
48 them had history of gastrointestinal symptoms or surgery. The study protocol was completed  
49 by 24 subjects (13 males, mean age 26 years, range 20-55). Two subjects were excluded  
50 because of poor tolerance during the first examination, one because of consent withdrawal  
51 and one because of recording failure.  
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Two manometric recordings were performed 2 to 7 days apart: one with HRM alone and one with HRM and PSM, in random order between May and September 2009.

HRM procedures were done with a 4.2 mm outer diameter solid-state assembly with 36 circumferential sensors spaced at 1-cm intervals (Sierra Scientific Instruments, Los Angeles, CA, USA). Before recording, transducers were calibrated at 0 and 300 mmHg using externally applied pressure. The manometry assembly was placed transnasally in a fasting subject and positioned to record from the hypopharynx to the stomach with approximately 3 intra-gastric sensors. The catheter was fixed in place by taping it to the nose.

Perfused-sleeve manometry examinations were realized with a multilumen assembly catheter fitted with a 6-cm sleeve (Dentsleeve PTY Ltd, Mui Scientific, Mississauga, Ontario, Canada) to monitor LES and esophageal pressure. The assembly was introduced through a nostril, swallowed and positioned so that pressure could be recorded from the fundus (2 cm below the sleeve), through LES (sleeve) and esophageal body (side holes 3, 8, 13, 18, and 23 cm proximal to the sleeve), to pharynx (side hole 28 cm proximal to the sleeve in order to detect swallowing). The Dentsleeve catheter was then fixed in place by taping it to the nose and infused at 0.5 ml/min using a low-compliance hydraulic capillary infusion system (Arndorfer Medical Specialties Inc., Milwaukee, WI) driven by a pressure head of nitrogen. The infusion system was connected to pressure transducers. Signals were recorded on a polygraph digitized, computer-processed, and stored using commercially available software (Polygram Net<sup>®</sup>, Medtronic Functional Diagnostics SAS, Skovlunde, Danmark).

To synchronize PSM and HRM the recordings were started together. A mark was added simultaneously by the examiner on both recordings every 15 minutes.

The recordings were performed in the semi-sitting position (45°). After 1 hour of recording in the fasting state, ten 5-ml water swallows were performed. Then the subjects ingested a standardized liquid meal (Clinutren 1.5, Nestle<sup>®</sup>, 400 ml, 600 kCal, 30% lipids).



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The meal had to be finished in 10 minutes. After ingestion of the liquid meal, the recordings were continued for 2 hours.

The study protocol has been approved by the appropriate ethical research committee (Comité de Protection des Personnes Sud Est III) and written informed consent was obtained from all subjects. This work was registered in ClinicalTrials.gov (NTC00931593).

### **Analysis of swallow-induced LES relaxations**

The EPT plots obtained from HRM alone recordings were analyzed using ManoView™ software (Sierra Scientific Instruments, Los Angeles, CA, USA). Basal LES pressure was measured during a 30-s period preceding the ten 5-ml water swallows. The swallow-induced LES relaxations were characterized by the nadir LES pressure and the 4-s integrated relaxation pressure (IRP), using the e-sleeve function of the ManoView™ software. The 4s-IRP reports the lowest mean LES pressure for 4 contiguous or non-contiguous seconds [17]. Nadir LES pressure and 4s-IRP were referenced conventionally to intra-gastric pressure. The duration of LES relaxation was defined as the time during which LES pressure was  $\leq 50\%$  of basal LES pressure. Isobaric contour and Smart Mouse tools were used to measure the nadir pressure and the relaxation duration.

### **Analysis of spontaneous LES relaxations**

The EPT plots obtained from the HRM alone recordings were reviewed independently by 2 observers: tLESRs events were firstly detected as reflux events (common cavity phenomenon with an abrupt increase  $\geq 5$  mmHg in intra-esophageal pressure) occurring with a spontaneous fall in LES pressure in the absence of swallowing 4 s before to 2 s after the onset of LES relaxation. The presence of swallowing was allowed if the LES relaxation duration was longer than 10 seconds in the absence of multiple swallows [8]. Each selected event was then reviewed by 3 observers to obtain a consensus.

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6 The spontaneous LES relaxations associated with common cavity were characterized  
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8 by the nadir LES pressure, the duration of LES relaxation and the 4-s IRP, with the same  
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10 method used for swallow-induced LES relaxations (Figure 1). For each spontaneous LES  
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12 relaxation, the basal LES pressure was established within the 10 seconds before the onset of  
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14 relaxation. The occurrence of crural diaphragm inhibition was noted. Esophageal shortening  
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16 was assessed by measuring the maximal elevation of the pressure band indicative of the  
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18 LES as proposed by Kwiatek et al. [14]. In instances of complete LES relaxation the final  
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20 location of the LES pressure band prior to complete relaxation or the first spatial location at  
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22 the end of the relaxation was used to measure maximal shortening (Figure 1).  
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25 The final motor event (swallow or secondary **contractile activity**) was noted for each  
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27 spontaneous LES relaxation. **Secondary contractile activity (measured as a contractile**  
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29 **activity  $\geq 3$  cm on the 20 mm Hg isobaric contour) was also noted in the absence of tLESR.**  
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31 The UES activity was also characterized during spontaneous LES relaxation. A UES  
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33 relaxation was defined as a break in the 20-mmHg isobaric contour at the level of the UES. A  
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35 UES contraction was defined as an increase of UES pressure from basal superior to 10  
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37 mmHg in the absence of UES relaxation. The UES pressure was measured within the 5  
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39 seconds before the onset of LES relaxation and within the LES relaxation using the smart  
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41 mouse tool.  
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44 Based on the comparison of swallow-induced and spontaneous LES relaxation  
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46 characteristics, HRM criteria of tLESRs detection were established. As tLESRs may occur  
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48 without reflux event these criteria were then applied independently by 2 observers (SR, FZ)  
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50 to characterize spontaneous LES relaxation episodes occurring without a common cavity as  
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52 tLESRs. In case of disagreement between the 2 observers the events were reviewed with a  
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54 third one (SBV) to obtain a consensus. The same criteria were applied to HRM recordings  
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56 obtained simultaneously to PSM recordings.  
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## Comparison of perfused-sleeve and high resolution manometry

The PSM and HRM tracings obtained from simultaneous recordings were reviewed independently by 2 observers. In case of disagreement, the events were reviewed by a third observer to obtain a consensus.

The identification of tLESRs on PSM tracings was done according to Holloway *et al* [8, 9]. The identification of tLESRs on HRM plots was performed according to the previously described criteria.

## Inter observer agreement

To assess inter observer agreement, 10 randomly selected recordings (from PSM tracings and HRM alone) were reviewed independently by 2 observers, using the above – mentioned criteria for both techniques. The inter observer agreement was calculated between the 2 observers.

## Statistical analysis

For each HRM criteria describing swallow-induced LES relaxation and tLESRs, a mean per subject was calculated and then criteria were expressed as median (5<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, 95<sup>th</sup> percentiles). Wilcoxon signed ranked test was used to compare continuous parameters and Chi square test for categorical parameters. **ROC curve analysis was performed to evaluate the performance of the individual criteria used to differentiate tLESRs from swallow-induced LES relaxations.**

The number of tLESRs detected per subject with each procedure (HRM alone, HRM with PSM) was expressed as median (interquartile range) and compared using paired t-test.

The concordance rate between PSM and HRM was assessed using kappa coefficient (95% confidence interval) [18]. For this analysis, each tracing was divided in 30-second windows and the presence or absence of tLESR identified by each procedure within each window was noted.

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6 Finally the inter-observer agreement was evaluated using kappa coefficient (95%  
7 confidence interval).  
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## 10 11 **Results**

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13 Simultaneous PSM and HRM recordings were available in 21 subjects (3 exclusions  
14 due to 1 impossible thermal compensation, 1 HRM recording failure and 1 misplacement of  
15 PSM probe) and recordings of HRM alone in 22 subjects (2 exclusions due to impossible  
16 thermal compensation).  
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### 22 23 **HRM description of swallow-induced LES relaxation and tLESRs**

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25 On HRM recordings performed with only the HRM probe, 183 swallows (median per  
26 subject 9 (7-10)) were analyzed and 239 spontaneous LES relaxations associated with  
27 common cavity were identified (median per subject 11 (5-15)). The characteristics of  
28 swallow-induced LES relaxation and tLESRs are described in Table 1, disclosing clear-cut  
29 differences for all parameters studied between both types of LES relaxation. **Among the 4**  
30 **parameters described, the most discriminant was the duration of LES relaxation, according to**  
31 **ROC curve analysis (Table 2 and Figure 2).**  
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42 Sixty additional spontaneous LES relaxations without common cavity (median per  
43 subject 2.5 (1-4)) were identified: all of them disclosed characteristics within the 5<sup>th</sup>-95<sup>th</sup>  
44 percentiles of the events associated with a common cavity. Therefore these 60 events were  
45 considered tLESRs.  
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51 A diaphragmatic inhibition was noted for all tLESRs. The median esophageal  
52 shortening was 1.3 cm (0.0, 0.0, 1.9, 3.0). The terminal motor events were secondary  
53 peristalsis in 53%, swallow in 39%, and absence of esophageal contraction in 8%. The  
54 terminal motor event was not different between tLESRs with or without common cavity  
55 ( $p=0.54$ ). **Of note, the majority (160/239, 67%) of secondary contractile activity events**  
56 **occurred after a tLESR.** UES pressure variations occurred more frequently in tLESRs  
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5 associated with common cavity than in tLESRs without (UES opening in 46.5% and 33.3%  
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7 and increased UES pressure in 40.0% and 18.3%, respectively,  $p < 0.01$ ).  
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### 10 11 **Comparison of PSM and HRM for the detection of tLESRs**

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13 During simultaneous recordings, the total number of tLESRs detected by PSM was  
14 significantly lower than the number of tLESRs detected by HRM (270 (median per subject 11  
15 (7-17)) vs. 352 (median per subject 15 (11-22) respectively,  $p < 0.01$ ).  
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19 Figure 3 represents the distribution of tLESRs among subjects. Significantly more  
20 tLESRs were detected with HRM than with PSM during the post prandial period (median per  
21 subject 12 (8-17) with HRM vs. 8 (5-12) with PSM;  $p < 0.01$ ). No difference was observed  
22 during the fasting period (median per subject 3 (2-5) with PSM vs 3 (1-5) with HRM;  $p = 0.80$ ).  
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29 Finally, 392 independent events compatible with tLESRs (97 during the fasting period  
30 and 295 during the post prandial period) were detected with PSM and/or HRM. As shown in  
31 Figure 4, a greater proportion of tLESRs were detected with HRM. Only 16% (64) of the  
32 events were detected by PSM alone (27% during the fasting period and 13% during the  
33 postprandial period). Most events detected by PSM only were due to a displacement of the  
34 perfused sleeve relatively to the EGJ position (69%). They corresponded to false positive  
35 tLESRs when analyzed simultaneously with HRM (Figure 5AD). For events detected by HRM  
36 only (149), 56% were also related to an incorrect positioning of the perfused sleeve relatively  
37 to the EGJ and corresponded to true positive tLESRs in HRM (Figure 5CE). Hypotensive  
38 LES was an important cause of discrepancies between both procedures (37% of events  
39 detected only by HRM) but in this situation it was not possible to determine if the event  
40 detected was truly a tLESR or not. The causes of discrepancies are detailed in Table 3.  
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55 The overall concordance rate between the 2 procedures was substantial ( $\kappa =$   
56 0.61 (0.56-0.67)). It was not different for the fasting and the postprandial periods ( $\kappa =$   
57 0.63 (0.53-0.73) and 0.61 (0.55-0.67) respectively). Noteworthy, differences existed among  
58 the subjects. The concordance was almost perfect ( $\kappa > 0.80$ ) for 4 subjects, substantial  
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(kappa 0.61-0.80) for 7 subjects, moderate (kappa 0.41-0.60) for 7 subjects, fair (kappa 0.21-0.40) for 2 subjects and slight for 1 subject (kappa < 0.21). For the 10 subjects with a kappa < 0.61, we observed 6 intra-gastric sleeve migrations after the meal (corresponding to esophageal shortening on HRM) (Figure 5) and 4 low postprandial LES pressure (< 8 mmHg).

### Reproducibility of HRM examinations

The number of identified tLESRs was not statistically different between the 2 HRM recordings (352 during simultaneous HRM and PSM session (median per subject 15 (11-22)) vs. 299 during HRM alone session (median per subject 13 (11-16)) (p=0.07) (Figure 3).

### Inter observer agreement

The inter observer agreement coefficients are given in Table 4.

The inter observer agreement was substantial or almost perfect for all subjects but one with HRM. The subject with moderate agreement had hypotensive LES (resting pressure < 10 mmHg). For PSM, large discrepancies were noted among the subjects: kappa coefficient varied from 0.15 (-0.23-0.52) to 0.82 (0.66-0.98).

## Discussion

As the main motor event associated with GER episodes, tLESRs occurrence has become a pivotal target for antireflux therapy. Therefore researchers need reliable tools to detect and characterize tLESRs. Considering the limitations of conventional perfused-sleeve manometry, mainly poor intra-observer agreement [9], our aim was to assess and validate the detection of tLESRs using HRM.

Using the common cavity as a marker of reflux event [19, 20] we were able to characterize tLESRs associated with reflux using HRM and define objective criteria for identifying these events. These objective criteria were then applied to identify tLESR

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6 occurring in absence of reflux event. Using the 5<sup>th</sup> or 95<sup>th</sup> percentiles of these criteria  
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8 (associated or not with common cavity) we showed that HRM allowed the detection of more  
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10 tLESRs than PSM and that the inter-observer agreement was definitely better with HRM than  
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12 with PSM.

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14 Our study reveals that the HRM characteristics of tLESRs are different in terms of  
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16 pressure and duration as compared to the criteria determined by Holloway et al. with  
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18 Dentsleeve perfused manometry [8]. However, the essential characteristics of tLESRs (i.e.  
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20 spontaneous long and profound relaxations associated with diaphragmatic inhibition) were  
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22 present on HRM-detected events. Technological issues may be responsible for these  
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24 discrepancies and this emphasizes the necessity to re-define HRM criteria for tLESR  
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26 detection. Differences in pressure values may exist between the different systems: for  
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28 example, taking into account the 20% of baseline LES pressure proposed by Holloway et al  
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30 [8] to define a LES relaxation would have resulted in an aberrant median value of swallow-  
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32 induced LES relaxation of 0 second in our subjects. Moreover, esophageal shortening  
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34 usually precedes tLESR [16]: a physical sleeve may record intragastric pressure (false LES  
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36 relaxation) while HRM and its electronic sleeve allows the LES pressure recording always at  
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38 the right position (Figure 4). Rohof et al. [21] recently published a comparison between HRM  
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40 and PSM applying the standard Holloway's tLESRs criteria [8, 9] to HRM recordings. This  
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42 study, performed with a different system (MMS®), found a higher rate of concordance  
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44 between the 2 techniques. Therefore, our criteria should also be tested on HRM recordings  
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46 obtained with different HRM systems.

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51 For the objective HRM definition of tLESRs, we decided to use the 4s-IRP to  
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53 characterize the LES relaxation as it has been demonstrated that the IRP quantifies LES  
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55 relaxation both in completeness and persistence [17], along more conventional parameters.  
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57 This metric was originally designed to assess swallow-induced LES relaxation and the  
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59 threshold of 4 seconds was determined as the best to discriminate patients with normal LES  
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relaxation and patients with achalasia. We believe that the 4s-IRP concept which consists of

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6 reporting the lowest mean LES pressure for 4 contiguous or non-contiguous seconds during  
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8 the deglutitive window can be applied to tLESR. In our subjects the 4s-IRP of tLESRs was  
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10 lower than the 4s-IRP of swallow-induced LES relaxations. **However, the most discriminant**  
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12 **parameter to distinguish tLESRs from swallow-induced LES relaxation was, besides the**  
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14 **presence or absence of swallow, the duration of the LES relaxation.**

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17 The crural diaphragmatic inhibition was consistent in events selected as tLESRs. This  
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19 characteristic is a good marker of tLESR [16] and is conserved in case of impaired LES  
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21 relaxation among patients with achalasia [14]. The UES pressure variations may also be  
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23 useful to identify tLESRs as they have been shown to be frequently associated with tLESRs  
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25 [11, 15]. **Secondary contractile activity was a frequent event terminating the tLESRs; however,**  
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27 **this activity cannot be regarded as a specific marker for reflux occurring after tLESRs, as**  
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29 **about 30% of these motor events were not associated with tLESRs.**

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32 HRM is a more sensitive tool than PSM since we detected significantly more tLESRs  
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34 with HRM, especially during the post prandial period. As shown in Table 3, this increased  
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36 sensitivity appears mainly driven by the almost continuous pressure measurements along the  
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38 HRM probe, thus overcoming the artifacts due to catheter movement or esophageal  
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40 shortening. The Dentsleeve displacement is responsible of not only false negative (Figure  
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42 5BD) but also false positive diagnosis of tLESRs (Figure 5CE). **Although this was not the**  
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44 **primary goal of our study, the results obtain here are also important because they establish**  
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46 **normal values (in term of frequency of tLESRs detected during fasting and after a**  
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48 **standardized meal), which are essential in order to compare with those of patients with**  
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50 **GERD or other esophageal disorders.**

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53 Our results show that the inter-observer agreement is definitely better with HRM than  
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55 with PSM. Reviewing pressure topography is easier for the human eye than reviewing  
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57 tracings [22]. EPT has been shown to facilitate the esophageal motility disorders diagnosis  
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59 [10]. Our data suggest a better sensitivity for the detection of tLESRs with HRM than with  
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PSM. To our knowledge, few studies reported the inter-observer agreement with PSM.



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6 Among experts, the concordance to identify tLESRs was 40 to 53% using the original criteria  
7 and 52 to 70% using the 2009 revised criteria [9]. In our hands, the inter-observer  
8 concordance was 25% with PSM and 72% with HRM.  
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12 One limitation of our study is the absence of pH-impedance detection of gastro-  
13 esophageal reflux to define the motor events associated with reflux. **Indeed, combining HRM**  
14 **and impedance would clearly establish the association between HRM-detected tLESrs and**  
15 **GER episodes (whether acidic, weakly acidic or weakly alkaline).** However, the common  
16 cavity phenomenon used in the present study is considered by experts as a manometric  
17 pattern of gastro-esophageal reflux, very specific though less sensitive than esophageal pH  
18 and/or impedance monitoring or fluoroscopy [19, 20]. A previous mechanistic study has  
19 shown that common cavity phenomenon was associated with the majority of tLESRs  
20 detected by HRM [16]. Finally, we characterized tLESRs in healthy subjects and further  
21 studies are mandatory to validate these criteria in GERD patients, and confirm the  
22 pathophysiological relevance of our criteria. In conclusion, HRM criteria for the objective  
23 definition of tLESRs have been established in the present study. HRM is more sensitive than  
24 PSM to detect tLESRs during prolonged LES pressure monitoring and provide a much better  
25 inter-observer agreement. Altogether, these results suggest that HRM should become the  
26 gold standard for detection and characterization of tLESRs, **and be now considered as part**  
27 **of the pharmacological evaluation of new drugs targeting tLESRs.**  
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## Acknowledgements and disclosures

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Sabine Roman, Frank Zerbib, Stanislas Bruley des Varannes, Kafia Belhocine and François Mion have received supplies from Sierra Scientific Instruments and Latitude Medical.

Frank Zerbib has received research funding from Nycomed.

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**Table 1:** Characteristics of swallow-induced and spontaneous LES relaxations associated with common cavity. A mean per subject was calculated for each criterion. Results are expressed as median (5<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, 95<sup>th</sup> percentiles).

	<b>Swallow-induced LES relaxation</b>	<b>Spontaneous LES relaxation associated with common cavity</b>	<b>p (Wilcoxon signed ranks test)</b>
Number of events per subject	9 (5,7,10,10)	11 (3, 5, 15, 24)	
Mean nadir pressure (mmHg)	5 (0, 2, 9, 11)	2 (0, 1, 4, 9)	<0.001
Mean 4-s IRP (mmHg)	8.0 (2.1, 3.6, 12.8, 14.3)	3.7 (0.6, 1.6, 6.0, 11.1)	<0.001
Mean % maximal relaxation	73 (19, 58, 84, 95)	80 (52, 71, 93, 100)	<0.004
Mean relaxation duration (s)	4.9 (0.1, 3.0, 6.8, 9.1)	13.0 (10.5, 11.7, 16.0, 18.6)	<0.001

**Table 2:** Results of the ROC curve analysis of the EGJ parameters used to differentiate tLESRs from swallow-induced LES relaxations.

	Duration of LES relaxation (s)	IRP-4s (mm Hg)	Nadir pressure (mm Hg)	% of LES relaxation
Cut-off value	> 8.9	≤ 6	≤ 1	> 96
Sensibility	88%	78%	55%	46%
Specificity	91%	61%	82%	90%
AUC	0.953	0.774	0.748	0.685
(95% CI)	(0.929-0.970)	(0.735-0.811)	(0.706-0.786)	(0.641-0.726)

**Table 3:** Causes of discrepancies between 213 events compatible with tLESRs detected by high resolution manometry (HRM) only or perfused sleeve manometry (PSM) only. The remaining 179 of the total 392 events were scored as tLESRs by both HRM and PSM.

	tLESRs by PSM only (n=64)	tLESRs by HRM only (n=149)
<b>True positive (%)</b>	<b>5 (8%)</b>	<b>84 (63%)</b>
- Intra-gastric perfused probe displacement due to post prandial esophageal shortening *	--	65 (44%)
- Perfused probe movement	--	18 (13%)
- Water perfusion artifacts	--	5 (3%)
- Pressure artifact on EGJ	5 (8%)	6 (4%)
<b>False positive (%)</b>	<b>55 (86%)</b>	<b>0 (0%)</b>
- Intra-gastric perfused probe displacement due to brief esophageal shortening ‡	18 (28%)	--
- Intrathoracic perfused probe displacement due to deep breath	12 (19%)	--
- Perfused probe movement	14 (22%)	--
- Missed swallow on PSM	8 (12%)	--
- Cough	3 (5%)	--
<b>Undetermined (%) because of hypotensive LES</b>	<b>4 (6%)</b>	<b>55 (37%)</b>

EGJ = esophago-gastric junction, IQR = interquartile range, LES = lower esophageal sphincter

\*See example figure 4CE

‡ Aspect of "pseudo-relaxation" on PSM tracings, see example figure 4AD

**Table 4:** Inter observer agreement for HRM performed alone and PSM.

	<b>kappa</b>	<b>95% IC</b>	<b>Inter observer agreement</b>
HRM	0.83	0.78-0.87	Almost perfect
PSM	0.38	0.27-0.50	Fair

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## Figures legend

Figure 1: Transient lower esophageal sphincter relaxation (tLESR) in HRM. The basal lower esophageal pressure is 28 mmHg. The isobaric contour corresponds to 50% of the basal LES pressure (14 mmHg, black line). This spontaneous LES relaxation is associated with the common cavity phenomenon, a relaxation of the upper esophageal sphincter and followed by secondary esophageal peristalsis. The nadir pressure of LES relaxation is 3 mmHg. The LES relaxation duration is measured within the period during which LES pressure is  $\leq$  50% of basal LOS pressure. This zone is delimited by the pink brackets. The 4-s integrated relaxation pressure (IRP) is measured within LES relaxation. It reports the lowest mean LES pressure for 4 non-contiguous seconds represented by the white boxes. All the pressures are referred to intragastric pressure. Esophageal shortening corresponds to the elevation of the pressure band indicative of the LES (dashed white lines) from the basal location to the final location prior to relaxation.

Figure 2: Comparison of ROC curves of the 4 HRM parameters (4-s IRP, LES relaxation duration, LES nadir pressure and % of LES relaxation) used to differentiate tLESRs from swallow-induced LES relaxations.

Figure 3: The box plots depict the distribution of the number of transient lower esophageal sphincter relaxations (tLESRs) detected per subject by each procedure (perfused-sleeve manometry (PSM) in white, simultaneous high resolution manometry (HRM) in light grey and HRM alone in dark grey) within the total recording period (on the left), the fasting period (in the middle) and the post prandial period (on the right). Each box has a height equal to the interquartile range; the horizontal bar indicates the median and the error bars represent the 5<sup>th</sup> and the 95<sup>th</sup> percentiles. The dots correspond to the extreme values. The total number of tLESRs detected per subject was significantly lower with PSM than with HRM ( $p < 0.01$ , paired

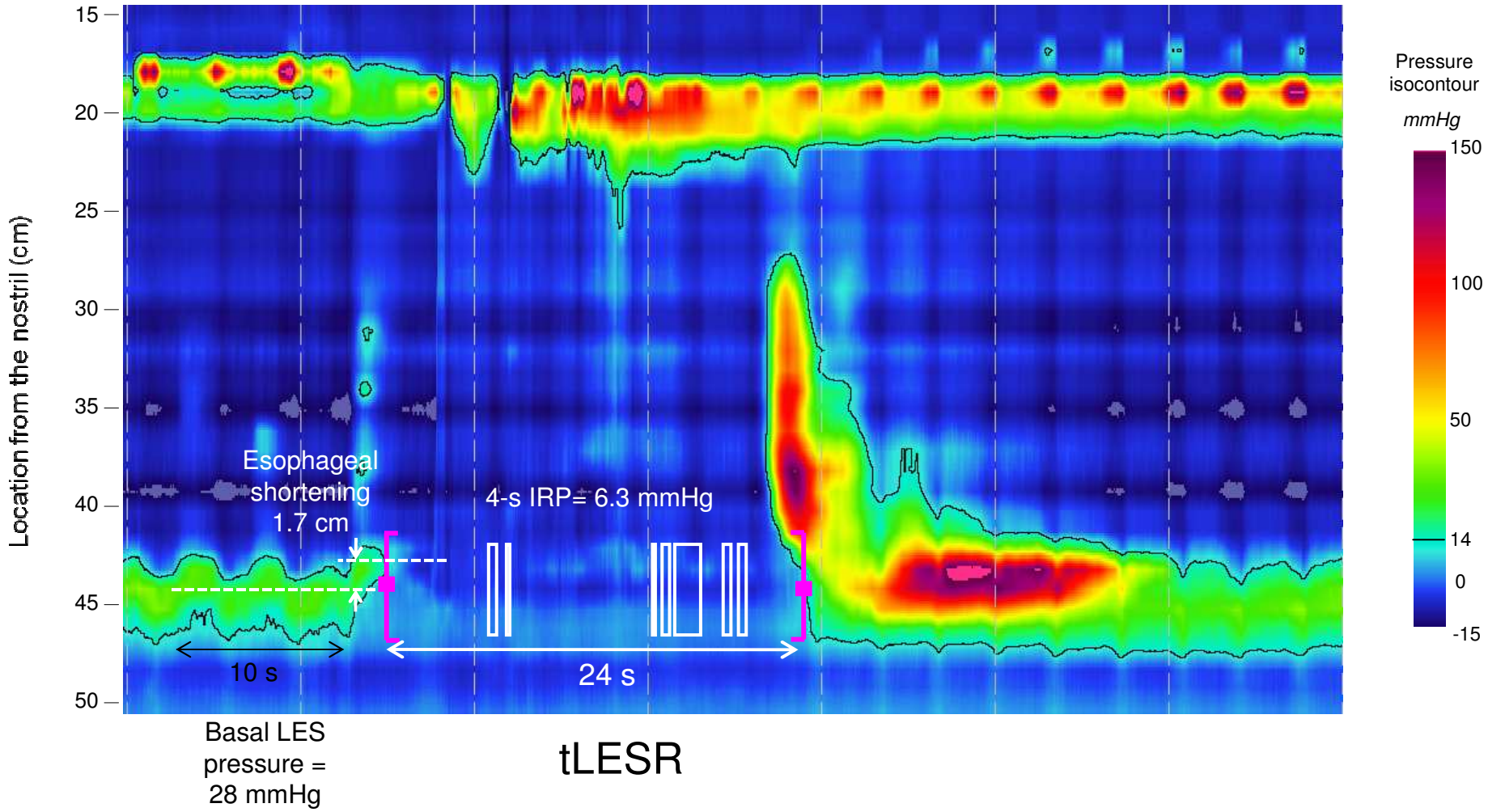
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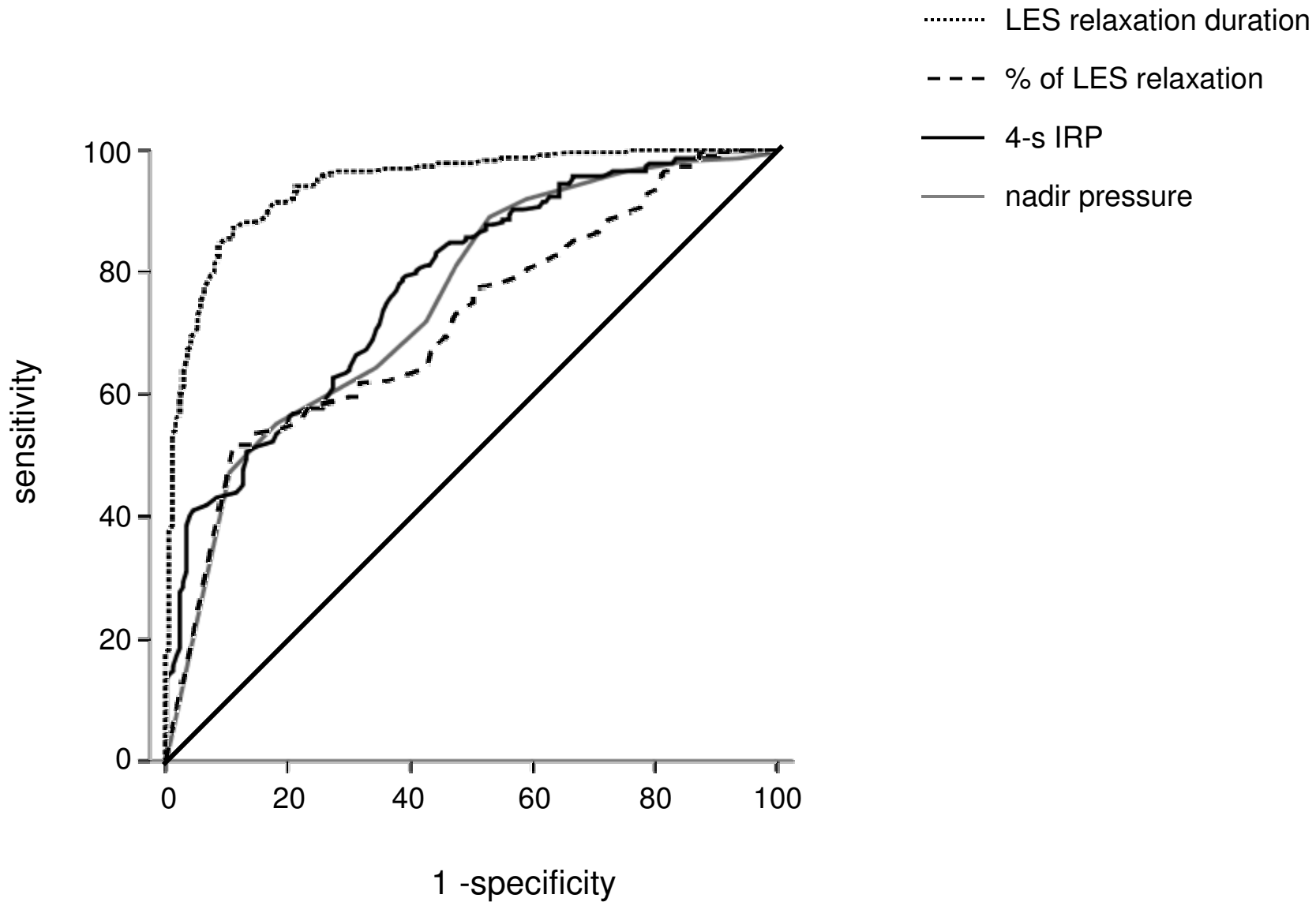
t-test). The number of post prandial tLESRs was also significantly lower with PSM than with HRM ( $p < 0.01$ , paired t-test). Finally, a greater number of fasting tLESRs is detected by simultaneous HRM compared to HRM alone ( $p < 0.01$ , paired t-test).

Figure 4: Proportion of tLESRs detected by perfused-sleeve manometry (PSM) and high resolution manometry (HRM) during the simultaneous recordings. Less than half of tLESRs were detected by both procedures (grey bars) in fasting and post prandial periods. A greater number of events were identified by HRM only (black) than by PSM (white).

Figure 5: Examples of discrepancies between perfused-sleeve manometry (PSM) (Panels A and B) and high resolution manometry (HRM) (Panels C, D and E). Panels A and C represent the same event with PSM and HRM as well as Panels B and E. A false positive tLESR in PSM is represented on Panel A. The event identified on PSM was a pseudo-relaxation as attested by the esophageal shortening on the corresponding esophageal pressure topography (EPT) (Panel C). On Panel B no event was detected with PSM whereas a tLESR was identified on the corresponding EPT (Panel E). This false negative tLESR in PSM (or true positive tLESR in HRM) was the consequence of intragastric perfused probe displacement. On EPT the position of the lower esophageal sphincter (LES) varied before (Panel D) and after the meal (Panel E). Note that the position of the upper esophageal sphincter (UES) remained unchanged. After the meal the perfused-sleeve was not located at the level of the LES but in the stomach.

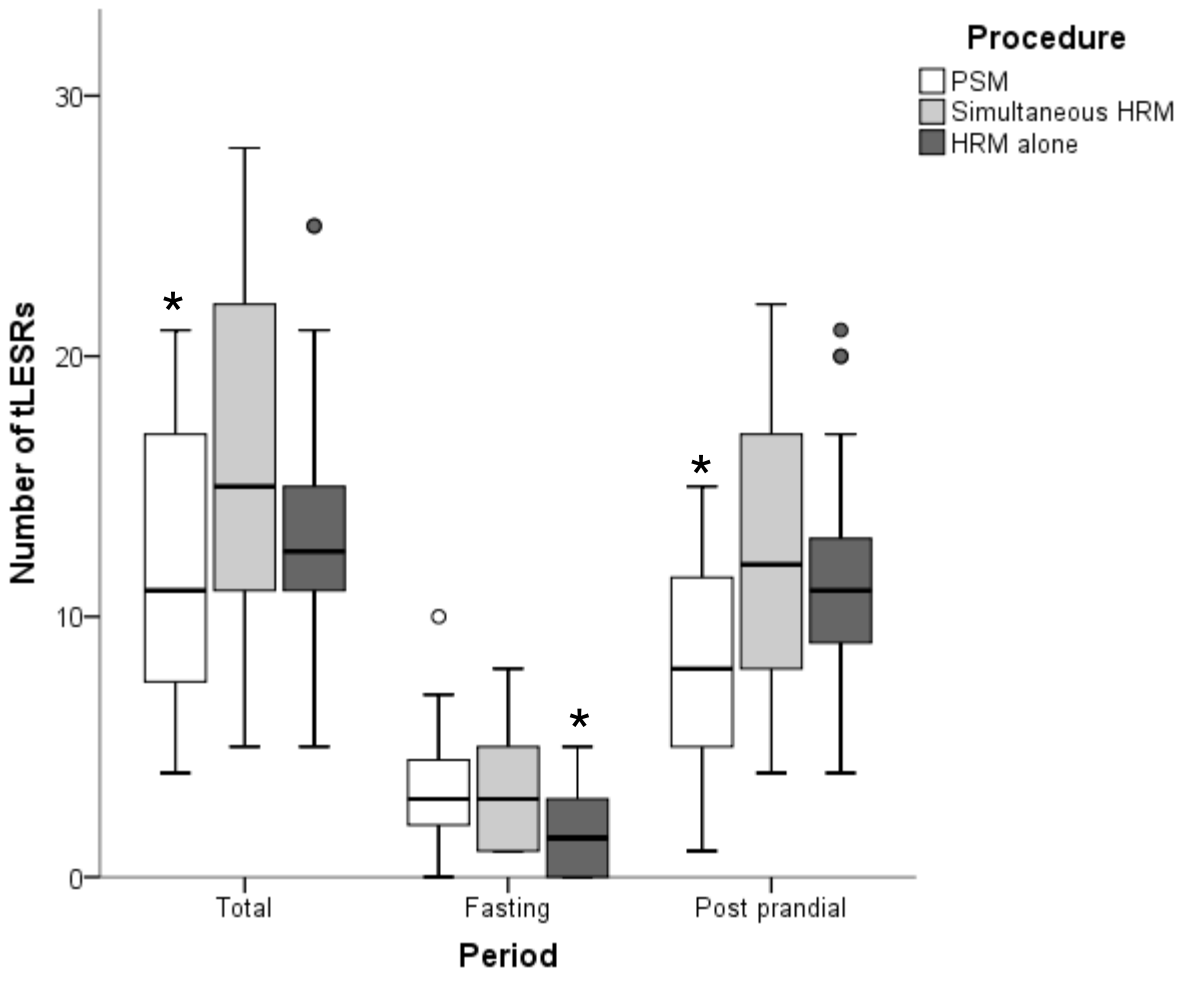
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\* p<0.01 vs simultaneous HRM

Figure 2

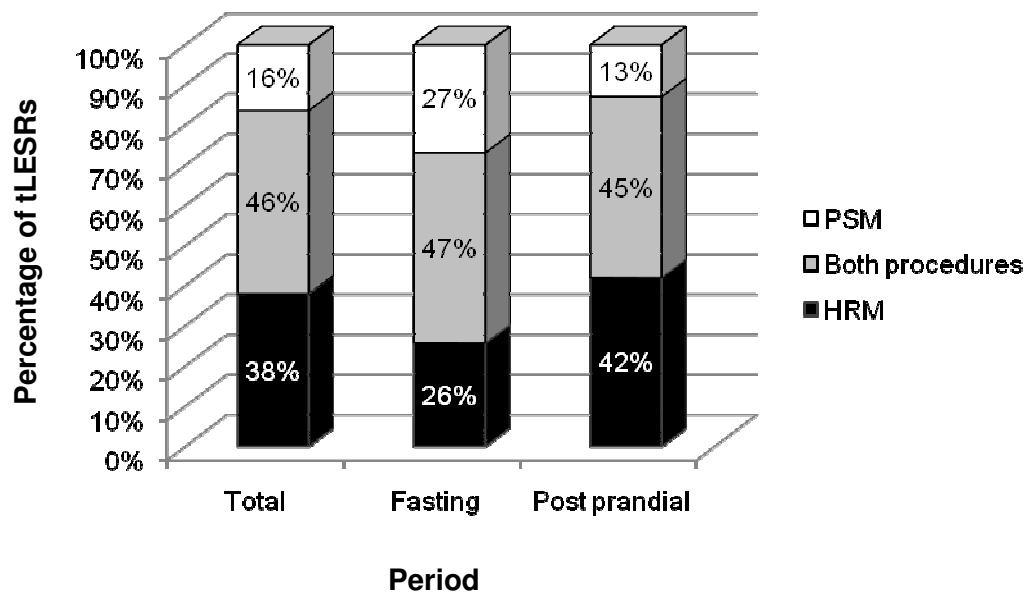
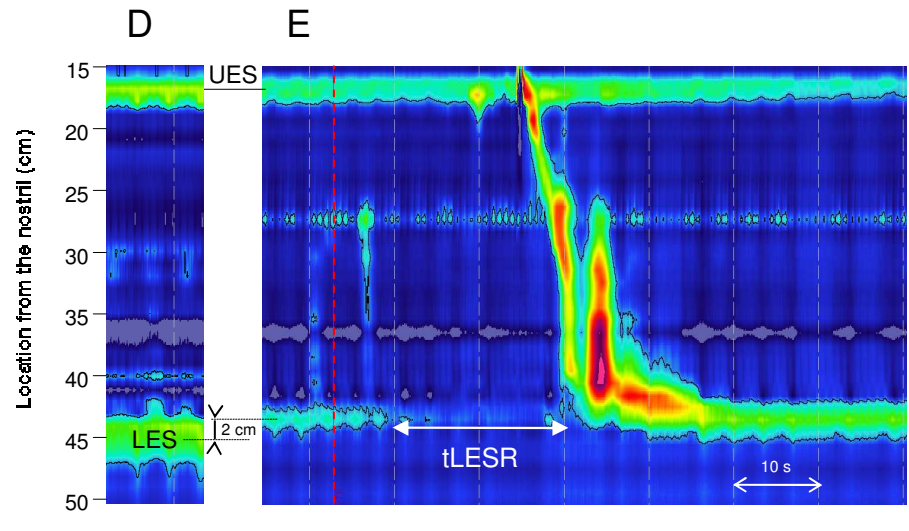
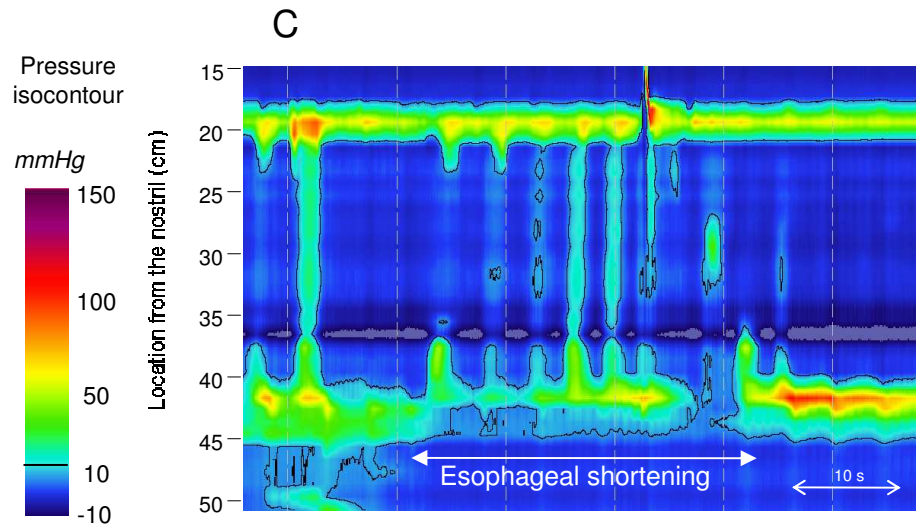
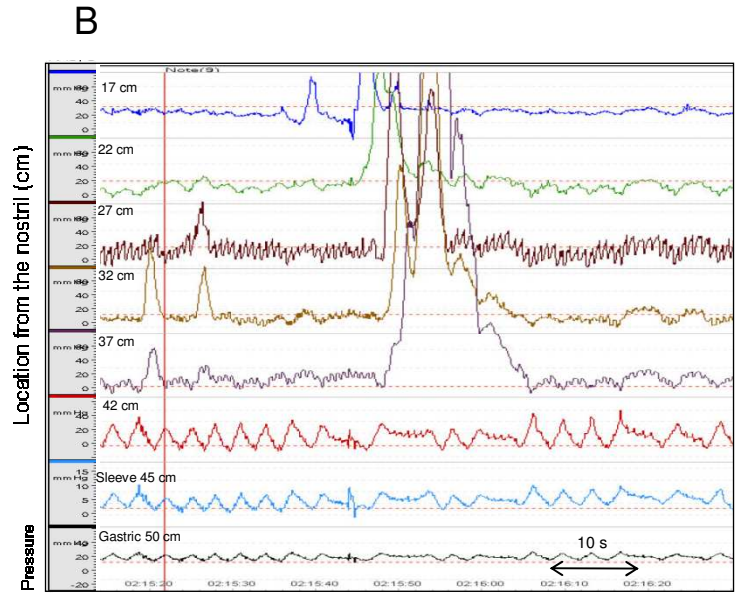
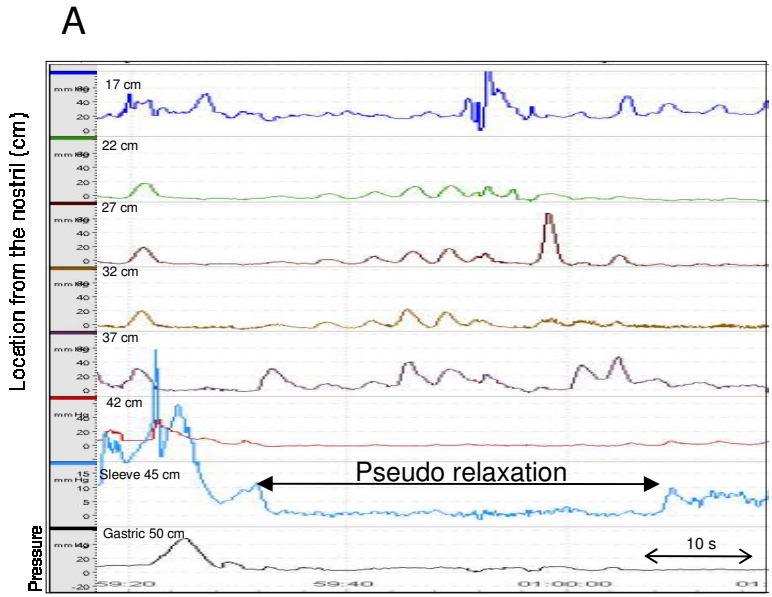


Figure 3

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Before the meal 60 min after the meal

Figure 4

# STARD checklist for reporting of studies of diagnostic accuracy

Section and Topic	Item #		On page #
TITLE/ABSTRACT/KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	5
METHODS			
<i>Participants</i>	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	6
<i>Test methods</i>	7	The reference standard and its rationale.	6
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	6
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	6
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	8
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	8
<i>Statistical methods</i>	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	9
	13	Methods for calculating test reproducibility, if done.	9
RESULTS			
<i>Participants</i>	14	When study was performed, including beginning and end dates of recruitment.	5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	5
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	9
<i>Test results</i>	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	5
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	NA: healthy volunteers
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	11
	20	Any adverse events from performing the index tests or the reference standard.	11



<i>Estimates</i>	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	NA
	22	How indeterminate results, missing data and outliers of the index tests were handled.	11
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	NA
	24	Estimates of test reproducibility, if done.	11
DISCUSSION	25	Discuss the clinical applicability of the study findings.	13

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