ABSTRACT

Background
Syndrome X is associated with signs and symptoms of ischemia without significant coronary artery obstruction. In these patients there is an increased perception of cardiac stimuli although the cause of this disorder is unknown.

Objective
The aim of this study was to analyze sensory nerve tracts involved in abnormal perception of pain in women with syndrome X.

Methods
This prospective study included 24 women: 12 with syndrome X; 6 with documented coronary heart disease, and 6 healthy controls. Lateral spinothalamic tract mapping by diffusion tractography was performed. The anatomic features (lines, voxels, and length) and physical features (fractional anisotropy, apparent diffusion coefficient, and diffusivity) of each tract were analyzed.

Results
The lateral spinothalamic tract was isolated in all patients. No differences were found in the physical characteristics of the tracts, but there was a significant difference in the number of voxels of the syndrome X group when compared to the other two groups (101.2 ± 46.9 vs. 83.2 ± 24 vs. ± 66 ± 16, p = 0.030), with a tendency towards a larger number of lines in each tract.

Conclusions
Differences in the anatomic characteristics of tracts were found in syndrome X patients with respect to healthy controls and coronary artery disease patients, with indemnity in the physical characteristics of the fibers. This is probably the first experimental study to show that it is possible to evaluate “in vivo” neurological tracts involved in pain transmission in syndrome X patients, opening a new field of research.

Key words
> Microvascular Angina - Ischemic cardiomyopathy - Magnetic Resonance Imaging – Physiology

Abbreviations
> ANC Angiographically normal coronary arteries
CA Coronary angiography
CD Coronary artery disease
CNS Central nervous system
FA Fractional anisotropy
PET Positron emission tomography
LST Lateral spinothalamic
MPS myocardial perfusion study
NMR Nuclear magnetic resonance
ROI Regions of interest

INTRODUCTION
Syndrome X is characterized by the presence of typical angina pain with absence of obstructive lesions in the epicardial coronary arteries, requiring a coronary angiography (CA) for its diagnosis (1). Although the pathophysiological mechanisms involved in this syndrome have not been fully elucidated, and there is still no consensus on the definition used (2), it is accepted that these patients must have proof of myocardial ischemia or coronary microvascular dysfunction in some diagnostic test (some authors have proposed “microvascular angina” as the best name for
syndrome X (3), and absence of any other secondary cardiac cause justifying pain / ischemia (4).

The prevalence of this disease is five times more frequent in women than in men, increasing in postmenopause (5). Most studies have shown that long term mortality is not higher than that of the general population adjusted by age and sex, (6) (7) although repeated episodes of pain result in assessments and diagnostic studies with increased costs for the health care system and deterioration of patient quality of life. (8).

The hypothesis that myocardial ischemia is the origin of syndrome X emerged from the first descriptions associating pain with ST segment alterations. Later, presence of irreversible deficiencies in myocardial perfusion studies (MPS), metabolic demonstration of ischemia, and endothelium-dependent and independent coronary vasodilation deficit, supported this theory. (9) These findings could be confirmed in some, but not in all, subsequent studies (10).

The other important hypothesis is the perception of cardiac pain (11): stimuli that produce no response in healthy controls or in patients with manifest coronary artery disease (CD) are capable of developing angina pain in patients with syndrome X (12). This finding has been tested with atrial and ventricular pacing (13), intra-atrial saline injection, and some intravenous drugs such as adenosine, dobutamine and dipyridamole. (14, 15)

The areas of the central nervous system (CNS) involved in pain perception have been determined in animal models and in subjects with anatomic lesions (16, 17). Coronary artery occlusion produces pain which is carried by the lateral spinothalamic (LST) tract to the thalamus (18), and from there to the primary somatosensory cortex (19). Studies with positron emission tomography (PET) in patients with coronary disease have found different areas of activation between syndrome X patients and healthy controls (17).

Despite these advances, the central mechanism that alters pain perception in syndrome X remains unknown, and there is not enough information about the anatomical characteristics of the afferent pathways that transmit it in man. The development of nuclear magnetic resonance (NMR) and the diffusion tensor tractography technique (derived from diffusion-weighted images (DWI)) have allowed the in vivo reconstruction of the two primary somatosensory pathways: the medial lemniscus and LST tract. (20)

The aim of this study is to identify the LST tract and its related thalamocortical fibers in a group of patients with syndrome X, and to assess its characteristics and integrity with respect to a group of patients with documented CD and healthy controls.

METHODS

Population
Twenty four patients were enrolled in this prospective study between January 2010 and April 2011 and were divided into three groups:

1) Twelve women with syndrome X: patients with exercise induced angina, with at least one episode of pain in the month prior to MRI, evidence of ischemia in MPS (at least two segments of a 17-segment model), a CA with an anatomically normal coronary arteries (ANC) and absence of any other cardiac / extracardiac cause that explained the symptoms.

2) Six women with CD: patients with chronic stable angina, not occurring at rest with at least one episode of pain in the month prior to MRI; with positive stress test (at least 2 MPS or echo stress segments out of a 17 segment-mod el) and evidence of >70% obstruction in at least one of the major epicardial arteries in a previous CA.

3) Six healthy control women: postmenopausal women with no known cardiac risk factors, and not receiving cardiac medications.

Exclusion criteria were: patients with prior psychiatric or neurological history (degenerative, vascular, structural, demyelinating diseases, etc.); patients with formal contraindications to receive NMR (mechanical devices such as pacemakers / prosthesis, claustrophobia or other phobias, etc.), and patients who did not sign the written informed consent prior to study initiation.

Technical Analysis:
Diffusion weighted images (DWI) were obtained using a Philips Achieva 1.5 T SENSE NMR System. These were analyzed using the software provided by the company (PRIDE, Philips Research Integrated Development Environment). Tractography was performed using a probabilistic method based on a multi-fiber model, with PRIDE software routines (Figure 1).

Two Regions of Interest (ROI) were outlined supported by volumetric T1-weighted sequences to define the LST tract according to the anatomy described in the brain atlas (21) and following the methods used in previous studies (22). The ROI 1 (Figure 2 A) was located at the level of the posterolateral region of the upper portion of the spinal cord, and the ROI 2 (Figure 2 B) was located at the level of the primary somatosensory cortex (S1). The tract was then verified frame by frame, manually excluding those fibers that were outside the tract. A neuroradiologist blind to the study (he/she did not know to which group each patient belonged) reviewed the images of each tract and its relationship to adjacent anatomical structures to ensure that it was the LST tract (Figure 3). In order to determine intraobserver and interobserver variability, tractography was performed by two operators: the first one (JPO) conducted two measurements separated by at least one month (to calculate the intraobserver variability), while the second operator (EAS) performed only one measurement which was compared with that of observer 1.

A total of 48 tracts (two tracts, right and left, for each of the study patients) were obtained. The LST tract is formed by afferent fibers which are added in each segment of the spinal cord, so that its structure progressively changes. For this reason its characteristics were conventionally measured from the superior section of the medulla oblongata, prior to thepons.

Image Analysis
Definitions: Variables of each tract were divided into two groups to facilitate analysis: anatomical variables (voxels, lines and length) and physical variables (fractional anisotropy (FA), apparent diffusion coefficient, radial and axial
The latter reflect the different degrees of water diffusion in the tract, which arises from integrating the diffusion in all the comprised (“tensors”) points, reflecting its integrity. Among them, FA is the most representative. When water diffusion occurs equally in all directions, it is called “isotropic” and adopts a spherical shape, but when there are barriers to the diffusion of water in some directions, as in living tissue, it takes the form of an ellipsoid, and is called “anisotropic”. The minimum is 0 if water diffuses with equal magnitude in all directions, with a maximum of 1 when it diffuses in a single direction.

RESULTS
The three groups were well balanced (Table 1), with a mean age of 53.5 ± 5.2 years. There were no differences in clinical variables, or in previous medications between the group of patients with syndrome X and the group of CD patients. As already described, none of the healthy women in the control group presented risk factors for CD or were receiving any cardiac medication.

Tractography was performed in all study patients. The intraobserver correlation coefficient was 0.89 for voxels, 0.85 for lines, and 0.94 for FA (all with p values <0.001), and the interobserver correlation coefficient was 0.88 for voxels, 0.83 for lines, and 0.93 for FA (all with p values <0.001).

The three groups presented different tract anatomy characteristics. The syndrome X group showed a significantly greater number of voxels (101.2 ± 46.9) compared to the coronary disease (83.2 ± 24) and the healthy control (66 ± 16) groups, with no significant differences between the latter two groups. The syndrome X group also presented a higher tendency in the number of lines, without difference in tract length (Figure 4 A).

There were no significant differences in the physical characteristics of the tracts: FA, apparent diffusion coefficient, and axial and radial diffusivity were similar in all three groups (Figure 4 B).

DISCUSSION
Some patients without evidence of obstructive coronary lesions or other underlying heart disease may have a typical angina pain episode associated with evidence of ischemia or microvascular dysfunction, a condition known as syndrome X (23). According to the definition used, this can be a very heterogeneous group of patients. Unlike previous studies, patients with the most accepted criteria were included in this study: only postmenopausal women (group where the
syndrome is more frequent), with at least one episode of typical angina pain in the last month, all of which had a positive test for myocardial ischemia (MPS in all cases). Hypertension and diabetes were considered as risk factors, and not “other heart conditions justifying the clinical presentation” as reported in the latest publications (24).

In the present study, using diffusion tensor tractography, afferent neural pathways responsible for cardiac pain transmission were identified, and it was possible to evaluate their physical and anatomical characteristics. Many previous studies have been able to reconstruct, identify and quantify somatosensory pathways with this technique.

In the nerve bundles, diffusion barriers are represented by cell membranes, axons and myelin (brain white matter) and any damage in these structures will be reflected in the analysis due to a decrease in fiber anisotropy.

The origin of increased sensitivity to cardiac pain may be due to any alteration in the pathway: from mechanoreceptors and chemoreceptors, cardiac afferents, spinal cord, thalamic filter, cortical connections to pain centers, and the whole complex modulation network acting at each level (25). In the brainstem, the LST tract is the main pain pathway, reaching the ventral posterolateral nucleus of the thalamus, and the related thalamocortical fibers are considered as part of the pathway (26).

Some studies have shown functional changes in cortical activity (represented by brain blood flow measured by PET) provoked by angina pain after induced ischemia in syndrome X patients, though with some methodological limitations (more women in the syndrome X group and predominantly more men in the CD group) (27). Furthermore, these areas show multiple functional and anatomical connections with other cortical and subcortical areas, encumbering valid conclusions. Because a stimulus may generate the activation of multiple central nuclei, we preferred to focus on the main pain pathway rather than on functional areas.

Patients with syndrome X in our study presented greater number of voxels with a tendency towards

<table>
<thead>
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<th>Characteristics</th>
<th>Coronary disease</th>
<th>Syndrome X</th>
<th>p</th>
</tr>
</thead>
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<tr>
<td>Age, years</td>
<td>57 ± 4.89</td>
<td>59.83 ± 6.34</td>
<td>0.102</td>
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<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>3 (50)</td>
<td>9 (75)</td>
<td>0.294</td>
</tr>
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<td>Diabetes</td>
<td>1 (16.7)</td>
<td>5 (41.7)</td>
<td>0.306</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>5 (83.3)</td>
<td>5 (41.7)</td>
<td>0.120</td>
</tr>
<tr>
<td>Smoking</td>
<td>3 (50)</td>
<td>3 (25)</td>
<td>0.294</td>
</tr>
<tr>
<td>Obesity</td>
<td>2 (33.3)</td>
<td>4 (33.3)</td>
<td>0.604</td>
</tr>
<tr>
<td>Previous treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>6 (100)</td>
<td>8 (66.7)</td>
<td>0.162</td>
</tr>
<tr>
<td>Thienopyridines</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
<td>0.333</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>2 (33.3)</td>
<td>6 (50)</td>
<td>0.437</td>
</tr>
<tr>
<td>ARBs</td>
<td>1 (16.7)</td>
<td>2 (16.7)</td>
<td>0.569</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>5 (83.3)</td>
<td>8 (66.7)</td>
<td>0.439</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>0 (0)</td>
<td>2 (16.7)</td>
<td>0.431</td>
</tr>
<tr>
<td>Statins</td>
<td>5 (83.3)</td>
<td>5 (41.7)</td>
<td>0.120</td>
</tr>
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ACE: angiotensin converting enzyme ARBs: angiotensin II receptor blockers

Fig. 4. Comparison between groups of lateral spinothalamic tract anatomical (A) and physical (B) characteristics.
higher number of lines in each tract. These findings allow us to speculate about a greater number of afferent fibers in the tracts conveying cardiac pain in these patients. Although we are unable to know whether this is due to a primary disorder or secondary to recurrent episodes of repetitive chest pain, the fact that patients with chronic stable angina (who as we pointed out should also have had pain in the last month) presented no differences with respect to healthy controls, argue in favor of the first hypothesis.

Our findings are not necessarily opposed to the previously mentioned studies: different types of stimuli can cause cardiac pain due to a greater number of afferent fibers, with activation of different regions of the cerebral cortex and increased brain blood flow. Although it is yet impossible to perform tractography of the efferent pathways innervating cardiac structures, if a similar finding were possible at the level of the sympathetic system (higher number of efferent fibers and adrenergic hyperactivity (28)), microvascular dysfunction observed in many of these patients could be explained.

There were no differences among the groups in the physical characteristics of the fibers. This phenomenon reveals tract integrity and indemnity, and discards the theory of some authors (29) of a primary fiber abnormality as probable cause of pain (infection, inflammation or demyelination).

If these findings were confirmed in a larger study, the current paradigm in clinical management of these patients could change, and we might perhaps focus on pain management through neuromodulation and use of new drugs, without neglecting control of risk factors and myocardial ischemia in patients with syndrome X.

Limitations
A limitation of the present work is the absence of induced pain during the process, which was not feasible due to the duration (approximately 20 minutes) of the NMR study. Furthermore, LST tract evaluation by diffusion tractography allows us to assess its anatomical and physical characteristics, but we cannot draw conclusions on impulse conduction (e.g. velocity of conduction or activation sequences at the level of the CNS).

We should note that in the characteristics of the evaluated fibers, those we call “physical characteristics” are more reproducible and have less technical influence than those we call “anatomical”. This observation and the small study sample caution us from drawing firm conclusions.

CONCLUSIONS
It is possible to evaluate “in vivo” the lateral spinotalamc tract anatomy in syndrome X patients. Although the indemnity of tract fibers appears to be preserved, the anatomical differences found in the group carrying this disease open a new field of hypotheses to be explored in the future.

RESUMEN
Evaluación del Haz Espinotalámico mediante Tractografía por Resonancia Magnética. Nuevos aportes en la Fisiopatología del Dolor en Pacientes con Síndrome X

Introducción
El Síndrome X se asocia a signos y síntomas de isquemia, sin obstrucción significativa de las arterias coronarias. Existe un aumento en la percepción de los estímulos cardíacos en estos pacientes, aunque se desconoce la causa de este trastorno.

Objetivo
Explorar los tractos nerviosos sensitivos involucrados en la percepción anormal del dolor en mujeres que sufren de Síndrome X.

Material y métodos
Estudio prospectivo que incluyó 24 mujeres: 12 con síndrome X; 6 con enfermedad coronaria documentada; y 6 controles sanas. Se realizó el mapeo del tracto espinotalámico lateral mediante tractografía por difusión. Las características anatómicas (líneas, voxels, longitud) y físicas (anisotropía fraccional, coeficiente de difusión aparente, difusividad) de cada tracto fueron analizadas.

Resultados
Se pudo aislar el haz espinotalámico lateral en todas las pacientes evaluadas. No hubo diferencias en las características físicas de los tractos, pero existió una diferencia significativa en el número de voxels de los 3 grupos a expensas del grupo síndrome X (101,2±46,9 vs. 83,2±24 vs. 66±16; p=0.030), con una tendencia a presentar mayor número de líneas en cada tracto.

Conclusiones
Existieron diferencias en las características anatómicas de los tractos de las pacientes con Síndrome X con respecto a controles sanos y pacientes con enfermedad coronaria, con indemnidad en las características físicas de las fibras. Este probablemente sea el primer estudio experimental en demostrar que es posible evaluar “in vivo” los tractos neurológicos involucrados en la transmisión del dolor en este grupo de pacientes, abriendo un nuevo campo de investigación.

Palabras clave > Síndrome X - Cardiopatía isquémica - Resonancia magnética nuclear - Fisiología.

Conflicts of interest
None declared.

REFERENCES