Background—Autonomic denervation is an alternative approach for patients with symptomatic bradycardia. No consensus exists on the critical targets and end points of the procedure. The aim of this study was to identify immediate end points and critical atrial regions responsible for vagal denervation.

Methods and Results—We enrolled 14 patients (50% men; age: 34.0±13.8 years) with cardioinhibitory syncope, advanced atrioventricular block or sinus arrest, and no structural heart disease. Anatomic mapping of ganglionated plexuses was performed, followed by radiofrequency ablation. Heart rate, sinus node recovery time, Wenckebach cycle length, and atrial-His (AH) interval were measured before and after every radiofrequency pulse. Wilcoxon signed-rank test was used for comparison. Significant shortening of the R-R interval ($P=0.0009$), Wenckebach cycle length ($P=0.0009$), and AH intervals ($P=0.0014$) was observed after ablation. The heart rate elevation was 23.8±12.5%, and the Wenckebach cycle length and AH interval shortening was 18.1±11% and 24.6±19%, respectively. Atropine bolus injection (0.04 mg/kg) did not increase heart rate further. Targeting a single spot of the left side (64% of the patients) or right side (36%) of the interatrial septum was observed to be responsible for ≥80% of the final R-R and AH interval shortening during ablation.

Conclusions—Targeting specific sites of the interatrial septum is followed by an increase in heart rate and atrioventricular nodal conduction properties and might be critical for vagal attenuation. The R-R interval, Wenckebach cycle length, and AH interval shortening, associated with a negative response to atropine, could be considered immediate end points of the procedure.

Key Words: atrial fibrillation • autonomic nervous system • bradycardia • denervation • reflex
WHAT IS KNOWN

- Ganglionated plexuses (GP) ablation is an evolving approach to treat symptomatic bradycardia in patients with cardioinhibitory vasovagal syncope associated with atrioventricular block or sinus arrest.
- Mapping and ablation of GP strategies are variable.
- The immediate end points at the conclusion of GP ablation have not been established.

WHAT THE STUDY ADDS

- The interatrial septum is a critical area, responsible for most of the parasympathetic tone modification observed after GP ablation. This data brings the perspective of performing ablation with a targeted interatrial septum approach.
- The R-R interval, Wenckebach cycle length, and atrial-His interval shortening, associated with an absence of an increased heart rate response to atropine could be considered immediate hard end points of the GP ablation procedure.

The study was approved by the institutional research committee, and all subjects provided informed consent.

Electrophysiological Study

The study was conducted with patients in a fasting state and under total intravenous anesthesia. The following was uniform heart disease. Exercise stress testing showed no ischemic ST abnormalities and normal chronotropic function. Thyroid function levels were normal. A negative polysomnogram excluded obstructive sleep apnea when suspected. Conventional electrophysiological study was performed before ablation and excluded sick sinus syndrome or primary dysfunction of the electric conduction system.

Electrocardiographic Diagnosis

Head-up tilt testing and 24-hour Holter recording were performed in all patients. The tilt testing protocol consisted of the following: 1, supine for 15 minutes before the test begins; 2, upright tilt to 70° for ≤20 minutes; and 3, oral 1.25 mg isosorbide dinitrate is administered if the test is tolerated and bradycardia or hypotension is not provoked. The table is then brought to the upright 70° position for a maximum of 20 minutes more. The diagnosis of cardioinhibitory syncope refers to patients with ≥3 episodes of loss of consciousness in the past 12 months, with no prodromal symptoms and ventricular pauses longer than 3 seconds during tilt table test. In accordance with the VASIS (Vasovagal Syncope International Study) classification of vasovagal syncope, these patients were considered as VASIS 2.15

Atriocentric block diagnosis refers to patients with episodes of ≥2 consecutive P waves blocked resulting in pauses longer than 3 seconds during 24-hour Holter recording. Sinus arrest was defined as transient cessation of atrial activity resulting in pauses longer than 3 seconds during 24-hour Holter recording. All patients had experienced at least 3 events (sycope or presyncope) in the past 12 months. These patients were considered as type 1 (asystole) based on the ISSUE classification (International Study on Syncope of Uncertain Etiology)16 of ECG-documented syncope.

Table tilt test was positive with a cardioinhibitory response in 4 patients (patient identification number [PIN] 1, 4, 11, and 13); 1 patient was diagnosed with transient sinus arrest (PIN 8), and the remaining 9 patients (PIN 2, 3, 5, 6, 7, 9, 10, 12, and 14) presented intermittent high-degree atrioventricular block in 24-hour Holter recording.

Electrophysiological Study

The study was conducted with patients in a fasting state and under total intravenous anesthesia. The following was uniform

### Table 1. Baseline Characteristics, Ablation Data, and Clinical Response

<table>
<thead>
<tr>
<th>PIN</th>
<th>Diagnosis</th>
<th>Age, y</th>
<th>Ablation Time, s</th>
<th>Procedure Time, min</th>
<th>Clinical Response</th>
<th>Critical Spot</th>
<th>SDNN Before the Procedure</th>
<th>SDNN 30 d After the Procedure</th>
<th>pNN&gt;50 Before the Procedure</th>
<th>pNN&gt;50 30 d After the Procedure</th>
<th>Follow-Up Time, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cardioinhibitory NMS</td>
<td>29</td>
<td>520</td>
<td>126</td>
<td>Remission</td>
<td>L</td>
<td>167</td>
<td>128</td>
<td>31.7</td>
<td>2.65</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>AVB</td>
<td>51</td>
<td>520</td>
<td>132</td>
<td>Remission</td>
<td>R</td>
<td>122</td>
<td>47</td>
<td>4.0</td>
<td>1.28</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>AVB</td>
<td>52</td>
<td>500</td>
<td>134</td>
<td>NR</td>
<td>R</td>
<td>103</td>
<td>54</td>
<td>6.4</td>
<td>0.39</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>Cardioinhibitory NMS</td>
<td>23</td>
<td>520</td>
<td>118</td>
<td>Remission</td>
<td>L</td>
<td>336</td>
<td>73</td>
<td>32</td>
<td>0.23</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>AVB</td>
<td>35</td>
<td>540</td>
<td>122</td>
<td>Remission</td>
<td>R</td>
<td>176</td>
<td>79</td>
<td>17.2</td>
<td>1.43</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>AVB</td>
<td>15</td>
<td>560</td>
<td>125</td>
<td>Remission</td>
<td>L</td>
<td>188</td>
<td>232</td>
<td>65</td>
<td>41.0</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>AVB</td>
<td>30</td>
<td>500</td>
<td>132</td>
<td>NR</td>
<td>L</td>
<td>172</td>
<td>155</td>
<td>28.7</td>
<td>31.8</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>Sinus arrest</td>
<td>41</td>
<td>420</td>
<td>94</td>
<td>Remission</td>
<td>L</td>
<td>182</td>
<td>65</td>
<td>22.0</td>
<td>4.0</td>
<td>16</td>
</tr>
<tr>
<td>9</td>
<td>AVB</td>
<td>17</td>
<td>430</td>
<td>99</td>
<td>Remission</td>
<td>R</td>
<td>187</td>
<td>92</td>
<td>34.8</td>
<td>6.8</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>AVB</td>
<td>18</td>
<td>500</td>
<td>98</td>
<td>NR</td>
<td>R</td>
<td>159</td>
<td>44</td>
<td>24.4</td>
<td>0.23</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>Cardioinhibitory NMS</td>
<td>54</td>
<td>480</td>
<td>94</td>
<td>Remission</td>
<td>L</td>
<td>205</td>
<td>117</td>
<td>23.0</td>
<td>1.1</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>AVB</td>
<td>22</td>
<td>470</td>
<td>108</td>
<td>NR</td>
<td>L</td>
<td>164</td>
<td>38</td>
<td>31.0</td>
<td>0.12</td>
<td>10</td>
</tr>
<tr>
<td>13</td>
<td>Cardioinhibitory NMS</td>
<td>47</td>
<td>450</td>
<td>98</td>
<td>Remission</td>
<td>L</td>
<td>185.2</td>
<td>68.0</td>
<td>19.7</td>
<td>1.47</td>
<td>9</td>
</tr>
<tr>
<td>14</td>
<td>AVB</td>
<td>42</td>
<td>450</td>
<td>95</td>
<td>Remission</td>
<td>L</td>
<td>289</td>
<td>43.2</td>
<td>11.3</td>
<td>2.13</td>
<td>9</td>
</tr>
</tbody>
</table>

Mean±SD

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Procedure Time, min</th>
<th>Response</th>
<th>Critical Spot</th>
<th>SDNN Before the Procedure</th>
<th>SDNN 30 d After the Procedure</th>
<th>pNN&gt;50 Before the Procedure</th>
<th>pNN&gt;50 30 d After the Procedure</th>
<th>Follow-Up Time, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.0±13.3</td>
<td>490±41</td>
<td>112±15</td>
<td>188.2±59</td>
<td>88.2±54</td>
<td>P=0.0018</td>
<td>25.0±14.9</td>
<td>6.7±12.8</td>
<td>P=0.0015</td>
</tr>
</tbody>
</table>

AVB indicates atrioventricular block; GP, ganglionated plexus; L, left side of the interatrial septum; NMS: neurally mediated reflex syncope; NR, nonresponder (patients who underwent permanent pacemaker implantation); PIN, patient identification number; pNN>50, percentage of sinus cycles differing from the preceding cycle by >50 ms during the 24-hour ECG recording; R, right side of the interatrial septum; and SDNN, SD of all normal R-R intervals in the 24-hour ECG recording.

*Ablation of the superior left GP and inferior left GP was obviated.*
in all patients: 2 mg/kg propofol, 3 μg/kg fentanyl, 3 μg/kg and 0.15 mg/kg cisatracurium in a single bolus dose were used for induction. Maintenance of anesthesia was performed with 50% O₂–50% air–2% sevoflurane mixture. Three femoral venous accesses were used to place multipolar catheters in the coronary sinus, His bundle position, right atrium (RA), right ventricle, and left atrium (LA; through trans-septal approach). Heparin was infused (10000–15000 IU bolus), and the activated clotting time, measured every 30 minutes, was maintained between 300 and 350 seconds. Intracardiac electrograms were displayed simultaneously on a multichannel recorder (EP tracer; Cardiotek, Maastricht, The Netherlands). Programmed atrial and ventricular stimulation ruled out sustained arrhythmias, primary conduction system dysfunction, and sick sinus syndrome.

**GP Mapping and Ablation**

Endocardial electroanatomic mapping of the right and left atria was performed with fast anatomic mapping technology/Carto 3 system ( Biosense-Webster, Inc, Diamond Bar, CA) in 10 patients (PIN 1, 2, 3, 4, 5, 6, 7, 8, 12, and 14). A multipolar catheter was used in 2 patients (PIN 12 and 14). EnSite Velocity (St. Jude Medical, St. Paul, MN) system was used in the remaining 4 patients (PIN 9, 10, 11, and 13). Specific atrium sites were empirically identified as GP by presumed anatomic location based on previous works (anatomic mapping):

1. In the LA: 1, the inferior right GP (posterior aspect of the inferior right pulmonary vein [PV]); 2, the anterior right GP (anterior aspect of the right PV common vestibulum); 3, the superior left GP (located between the LA roof and the left superior PV ostium); 4, the inferior left GP (between the posterior wall and coronary sinus ostium); and 5, the septal aspect of the superior vena cava junction (opposite to the LA GP tags).

We used a 3.5-mm irrigated tip catheter (Navistar ThermoCool; Biosense-Webster) or a 4-mm irrigated tip catheter (Therapy Cool Path, St. Jude Medical Inc) to deliver radiofrequency energy (50°C, 20–30 W, for 30–60 seconds) with a 20 mL/min irrigation flow.

The order for ablation was prespecified. It started by targeting the LA GPs: beginning with the inferior right GP and superior right GP. Ablation of the superior left GP and inferior left GP of the LA was obviated in the last 11 patients included (PIN 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14), for simplification purposes. Finally, the RA (posterior right and then the superior right) plexuses were targeted.

**Immediate Autonomic Evaluation**

Electrophysiological measures were consistently recorded throughout the study, immediately before and after every radiofrequency pulse applied to the prespecified sites: Sinoatrial node function was evaluated by measuring the heart rate (HR) and mean corrected sinus node recovery time after 60-second pacing with 3 different cycle lengths: 600, 500, and 430 ms.

Atrophicventricular node function was evaluated by measuring the atrial-His (AH) interval and Wenckebach cycle (WC) length (stimulation cycle length wherein Wenckebach atrophicventricular block occurs). HR variability (SDNN and pNN>50) was evaluated before and 30 days after the procedure. SDNN was the SD of all normal R-R intervals in the 24-hour ECG recording, and pNN>50 was the percentage of sinus cycles differing from the preceding cycle by >50 ms during the 24-hour ECG recording.

**Statistical Analysis**

HR variability measures (SDNN and pNN>50) before and 30 days after the procedure were compared to verify clinical autonomic attenuation.

The HR, sinus node recovery time, WC length, and AH interval were measured before and after every GP ablation and tested as possible physiological end points. Wilcoxon signed-rank test was used for comparison between pre- and postablation measures. A value of

| Table 2. Electrophysiological Characteristics Before and After Ablation |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                  | R-R Before Ablation, ms   | R-R Final, ms               | Short %                     | AH Interval Before Ablation, ms | AH Interval After Ablation, ms | Short %                     | WC Length Before Ablation, ms | WC Length After Ablation, ms | Short %                     | SNRTc Before Ablation, ms   | SNRTc After Ablation, ms    | Short %                     | AH indicates atrial-His; PIN, patient identification number; Short %, interval shortening (%); SNRTc, corrected sinus node recovery time; and WC, Wenckebach cycle. |
Results
We enrolled 14 consecutive patients (50% men; age: 34.0±13 years) in this study. The mean total duration of the procedure was 112±15 minutes, the total fluoroscopic time was 16±7 minutes, and the mean total ablation time was 490±41 seconds per patient (Table 1). We defined procedure termination when all prespecified anatomic GP tags had been ablated. No intraprocedural complications were observed.

A significant and immediate shortening of the R-R interval (P=0.0009), WC length (P=0.0009), and AH interval (P=0.0014) was observed after ablation (Table 2), although the corrected sinus node recovery time did not present significant changes (P=0.19). HR elevation was 23.8±12.5%, and the WC length and AH interval shortening was 18.1±11% and 24.6±19%, respectively, compared with initial values (Figure 1). Atropine bolus injection (0.04 mg/kg) did not increase HR further, demonstrating effective vagal tonus modification (Table 2).

Targeting a rather discreet portion of the interatrial septum triggered the major impact on the final HR and AH intervals during the ablation. Radiofrequency energy applied to a single spot on the left side of the septum, likely related to the anterior right GP and inferior right GP (Figure 2; 64% of the patients), or on the right side of the interatrial septum, opposite to the LA GP tags (Figure 3; 36% of the patients), was responsible for ≥80% of the final R-R and AH interval shortening.

Certain GP sites have been observed to trigger differential effects on the sinoatrial and atrioventricular nodes (Figure 4): PIN 5 presented significant shortening of AH interval (228–102 ms) but no R-R interval changes after ablation of the RA posterior GP. R-R shortening (1280–670 ms) was only observed after ablation of the RA superior GP. Similarly, PIN 12 and 14 demonstrated a differential effect of LA right superior GP and LA right inferior GP ablation on the sinoatrial and atrioventricular nodes, respectively, although this behavior was not uniformly detected during the procedures of the remaining patients.

During a mean follow-up time of 22.5±11.3 months (range, 9–38 months), 10 patients (71.4%) presented significant clinical and ECG improvement (PIN 1, 2, 4, 5, 6, 8, 9, 10, 12, 14).
with no syncope recurrences or bradycardia observed during daytime (transient second-degree atrioventricular block was still detected, exclusively at night, during follow-up Holter recording of PIN 2, 6, and 8). The remaining 4 patients (28.6%), PIN 3, 7, 10, and 12, although presenting R-R interval, WC length, and AH interval shortening similar to the rest of the cohort (Table 2), had syncope recurrence or symptomatic bradycardia and underwent pacemaker implantation (symptomatic atrioventricular block was detected by telemetry immediately after ablation of PIN 3, 7, and 10, and the device was implanted before discharge; PIN 12 presented with syncopal atrioventricular block 60 days after discharge, refused a second ablation, and a pacemaker was then implanted.). No significant atrial arrhythmias were observed during follow-up.

Discussion
The main findings of this study are the following:

1. The ablation of GP located on the right and left sides of the interatrial septum is responsible for most of the parasympathetic tone modification observed during denervation, suggesting that targeting this area and sparing other atrial regions might be enough to treat bradycardia-related symptoms. This conservative denervation strategy could possibly result in less extensive ablation and reduced procedure duration.

2. We also identified immediate clinical and electrophysiological end points (WC length and AH interval shortening, HR increment, and negative response to atropine) that directly indicate vagal tone attenuation and could be used instead of arbitrary surrogate end points, such as evoked reflex abolition or elimination of atrial fractionated potentials.

3. Finally, we observed that 4 patients (28.6%, all with transient advanced atrioventricular block) did not present clinical improvement after ablation and required pacemaker implantation.

Endocardial GP ablation has been increasingly used to treat severe vagal-related arrhythmias. Although the guidelines indicate pacemaker implantation for cases of symptomatic bradycardia and atrioventricular block, the fact that these patients are mostly young and otherwise healthy individuals encourages a more conservative approach.

The intrinsic cardiac nervous system forms a complex neural network composed of GP and interconnecting axons. Larger GPs observed close to the PV ostia serve as autonomic integration centers modulating cardiac excitability. This widely distributed structure cannot be fully targeted, and a comprehensive and selective approach, meant to promote attenuation instead of a total vagal blockade, is required.

Several authors reported a significant clinical impact after ablation of the interatrial septum, suggesting that this
area might contain the main vagal pathways to sinoatrial and atrioventricular nodes among other atrial regions. Occasionally, selective innervations to sinoatrial and atrioventricular nodes may be possibly identified by targeting specific sites,22–24 such as those verified during ablation of PIN 5, 12, and 14.

In the present study, we observed that radiofrequency energy applied on the interatrial topography triggers the most important electrophysiological changes expected during denervation. The identification of the interatrial septum as a critical area brings the perspective of performing ablation with a conservative septum-restricted approach.

It is noteworthy to mention that the procedure started with radiofrequency pulses targeting the LA GPs in all patients. This first step resulted in significant R-R interval, AH interval, and WC length shortening in 64% of the patients, but the remaining 36% of the patients (PIN 2, 3, 5, 9, and 10) only presented autonomic changes after targeting the RA GPs. Although the clinical impact of an exclusive RA or LA ablation was not studied or compared in this work, the fact that more than one third of the patients required a combined RA and LA approach indicates that this is probably the most adequate technique.

Instead of using surrogate end points, our results propose hard physiological end points. High-frequency stimulation reflex abolition is probably the most broadly used end point in previous works2–6,8,9,11,12 but requires specific equipment, causes atrial electric disturbance, and might have low sensitivity, appearing not to be effective in long-term clinical follow-up.5,12 Recent works have adopted combined end points (high-frequency stimulation abolition plus fractionated electrogram elimination)12 or a broader anatomic-based ablation strategy,25 resulting in nonspecific larger scar areas.

Although Cui et al26 could not verify this effect in experimental studies (with open-chest epicardial approach and aggressive lesions, destroying both sympathetic and parasympathetic elements), the significant enhancement of the sinoatrial and atrioventricular node functions after endocardial GP ablation.
has been consistently demonstrated. We might question whether AH shortening reflects autonomic tone modification or is merely because of the R-R change, but the differential effects of certain GP sites on the sinoatrial and atrioventricular nodes (as observed in PIN 5, 12, and 14) suggest that R-R and AH intervals behave independently. Therefore, immediate evaluation of HR, AH interval, and WC length could be reliably used to identify adequate vagal attenuation.

Last, we reported that 4 of the 14 studied patients (28.6%) presented syncope or presyncope and persistence of severe bradycardia after ablation, despite atrioventricular and sinoatrial nodal function enhancement, and underwent pacemaker implantation (all within 60 days after ablation).

The recurrence rates vary between 0% and 27%, based on previous studies that evaluated the effect of vagal denervation on patients with symptomatic bradycardia. Persistence of symptoms and severe bradycardia were observed only in the group of patients with advanced atrioventricular block in this cohort, although the limited size of the cohort prevents establishing a definite correlation.

The incidence of nonresponders may have several explanations. Late vagal tonus recovery after denervation with different techniques is an important, well-demonstrated issue. Ripplinger et al describes the neuroanatomy of the atria as abundant intrinsic ganglia more widely distributed than previously thought. Therefore, a significant portion of the vagal
innervation after radiofrequency lesions may possibly remain stunned but still functional. In that case, a redo procedure might be useful.

Another explanation for clinical failure lies in the fact that the atrial ganglia serve as autonomic integration centers. Some patients might have multiple vagal pathways to the sinoatrial and atrioventricular nodes, and others might become active by destroying an input. This could have been the recurrence mechanism of PIN 7, who presented immediate R-R and AH interval shortening, associated with atrial septum is often followed by a significant increase in HR and atrioventricular nodal conduction properties and might be critical to achieve vagal tonus attenuation. The R-R interval, WC length, and AH interval shortening, associated with a negative response to atropine, could be considered the main immediate end points of the procedure.

Sources of Funding
Dr Hachul receives support from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)–2012/00559-5.

Disclosures
None.

References


Targets and End Points in Cardiac Autonomic Denervation Procedures
Esteban W. Rivarola, Denise Hachul, Tan Wu, Cristiano Pisani, Carina Hardy, Fabrizio Raimundi, Sissy Melo, Francisco Darrieux and Mauricio Scanavacca

*Circ Arrhythm Electrophysiol.* 2017;10:e004638
doi: 10.1161/CIRCEP.116.004638

*Circulation: Arrhythmia and Electrophysiology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/10/2/e004638

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation: Arrhythmia and Electrophysiology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation: Arrhythmia and Electrophysiology* is online at:
http://circep.ahajournals.org/subscriptions/