Diagnostic Utility of Mechanical, Pharmacological and Orthostatic Stimulation of the Carotid Sinus in Patients With Unexplained Syncope

Carlos A. Morillo, MD,‡ Maria E. Camacho, MD,‡ Mark A. Wood, MD, FACC,* David M. Gilligan, MD, FACC,† Kenneth A. Ellenbogen, MD, FACC*

Richmond, Virginia and Bucaramanga, Colombia

OBJECTIVES
The purpose of the present study was to systematically evaluate the diagnostic utility of mechanical, pharmacological and orthostatic stimulation of the carotid sinus in a consecutive series of patients with recurrent unexplained syncope.

BACKGROUND
Carotid sinus hypersensitivity (CSH) is an infrequently recognized cause of recurrent unexplained syncope usually diagnosed by carotid sinus massage (CSM) in the supine position. The diagnostic utility of systematic assessment of mechanical, pharmacological and orthostatic stimulation of the carotid sinus has not been clearly established.

METHODS
Eighty consecutive patients (63 ± 12 years) with a history of recurrent unexplained syncope (mean episodes: 6 ± 3); 30 age-matched controls (65 ± 14 years) and 16 patients (59 ± 12 years) with syncope not related to CSH were studied. Pharmacological stimulation of the carotid sinus was achieved by randomly administering bolus injections of nitroprusside and phenylephrine. Mechanical stimulation of the carotid sinus was performed by CSM applied for 5 s in the supine position and after 2 min at 60°. A 60° low-dose isoproterenol head-up tilt test (HUTT) was also performed for a total duration of 30 min.

RESULTS
Carotid sinus hypersensitivity was elicited by CSM in the supine position in seven (8.7%) patients, two (6.6%) controls and one (6.3%) patient with syncope unrelated to CSH, compared with 48 (60%) patients, two (6.6%) controls and one (6.3%) syncope unrelated to CSH patient after 60° HUTT, increasing the diagnostic yield by 51%. Baroreceptor gain was significantly reduced in the CSH group. Head-up tilt test was positive in 12 (25%) patients with CSH, two (6.6%) controls and two (12%) with documented syncope but not positive in any of the patients in which syncope remained unexplained. Diagnostic accuracy was enhanced by 38% (31% supine vs. 69% upright) when CSM was performed at 60°.

CONCLUSIONS
CSH was documented in 68% of patients, 8.7% in the supine position and 60% in the upright position. Sensitivity was increased by 51%, and diagnostic accuracy was enhanced by 38% by performing CSM in the upright position. Decreased baroreceptor gain was documented and may play a role in the pathophysiology of CSH. (J Am Coll Cardiol 1999;34:1587–94) © 1999 by the American College of Cardiology

The incidence of carotid sinus hypersensitivity (CSH) as a cause of unexplained syncope is unclear and may be underestimated particularly in elderly patients (1–6). Morley et al. (1) estimated that the number of newly diagnosed cases of CSH associated with syncope or presyncope is 35 per million population per year. Carotid sinus hypersensitivity is usually diagnosed by eliciting significant bradycardia and/or hypotension associated with presyncope or syncope, during carotid sinus massage (CSM) usually performed in the supine position. However, the relevance of provoking significant bradycardia/hypotension during CSM may be limited by the lack of standardization of this method. Similarly, the effect of orthostatic stress during carotid sinus stimulation has not been systematically assessed. Given the fact that CSH is a form of neurally mediated syncope, it appears pertinent to assess the effects of orthostatic stress during CSM in patients with recurrent unexplained syncope.

The purpose of the present study was to systematically assess the diagnostic utility of mechanical, pharmacological and orthostatic stimulation of the carotid sinus of patients with unexplained syncope and compare it with a control
group with no previous history of syncope and a group with documented causes of syncope not related to CSH.

**METHODS**

**Study population.** Eighty consecutive patients aged 46 to 85 years (mean 63 ± 12 years) with a history of two or more syncopal episodes in the preceding 6 months and 30 age-matched controls 48 to 89 years (mean 65 ± 14 years) with no history of syncope or presyncope were studied. Additionally, 16 subjects aged 31 to 74 years (mean 59 ± 12 years) with syncope not related to CSH (12 ventricular tachycardia/ventricular fibrillation [VT/VF], two complete AV block, two severe sinus node dysfunction) underwent the same study protocol. All patients underwent complete physical and neurological examination as well as 12-lead electrocardiogram (ECG), 24- to 48-h ambulatory ECG monitoring and a two-dimensional echocardiogram. Additionally, all 16 subjects with syncope not related to CSH underwent complete electrophysiologic evaluation. Verbal and written informed consent was obtained in all patients.

**Carotid sinus stimulation protocol.** All subjects were studied in the postabsorptive state between 8:30 AM and 12:00 noon. An intravenous line was inserted, and 5% dextrose in normal saline was begun at a rate of 30 ml/h. Patients were allowed 15 min for stabilization and data acquisition. Continuous noninvasive assessment of blood pressure (Omeha 2300; Finapress) and ECG lead II were recorded by CAFTS system (MEIKRO, Finland) and simultaneously saved on the hard drive of a personal computer for further data analysis.

**Pharmacological stimulation.** Pharmacological stimulation of the carotid sinus was achieved by randomly administering a bolus injection of 100 µg of nitroprusside followed after 60 s by 150 µg of phenylephrine (7). Five to 10 min were allowed for restoration of baseline parameters, and the procedure was repeated after reversing the order of the bolus injections. The maximum change in heart rate and systolic and diastolic blood pressure with both vasoactive agents was calculated and compared within groups. Baroreceptor reflex slope was calculated by plotting each R-R interval as a function of the preceding systolic blood pressure, and beat-by-beat analysis was performed when R-R interval changed. A least-squares-fit linear regression was performed, and reflex control of heart rate was expressed as the slope of the linear regression line. Only slopes with regression coefficient ≥ 0.7 were accepted for analysis. Slopes were calculated for both nitroprusside and phenylephrine infusion as well as for the whole range of R-R intervals and systolic blood pressures during both infusions (7).

**Mechanical stimulation.** Five minutes for recovery was allowed after the pharmacological interventions, and CSM was performed by gently applying pressure over the carotid sinus for a period of 5 s initially on the right side. This procedure was repeated at least twice on each side, and beat-to-beat changes in heart rate and blood pressure were simultaneously recorded. Another 5 min was allowed for recovery, and the patients were subsequently placed on the upright position at 60°. Two minutes after assuming the upright position, CSM was repeated as previously described. If either presyncope or syncope was elicited, the patient was promptly returned to the supine position and allowed to recover spontaneously.

**Head-up tilt protocol.** Head-up tilt test (HUTT) was performed in all patients regardless of the outcome of the carotid sinus stimulation protocol. The subjects were placed in the upright position at 60° for 15 min. If neither presyncope nor syncope was not induced, an isoproterenol infusion was started at a rate of 1 µg/min and titrated until an increase in sinus cycle length of 25% was achieved. The maximum dose infused did not exceed 3 µg/min, and the total duration of the protocol was 30 min. Detailed description and validation of this HUTT protocol has been previously reported (8).

**Definitions.** Carotid sinus hypersensitivity was defined as a fall in systolic blood pressure ≥ 50 mm Hg and/or bradycardia ≥ 40 beats/min with or without asystole of 3 s or more (1), associated with syncope or presyncope that resembled the clinical presentation. Carotid sinus hypersensitivity was further classified as: 1) cardioinhibitory type, asystole ≥ 3 s not preceded by significant drop in blood pressure; 2) vasodepressor type, fall in systolic blood pressure ≥ 50 mm Hg associated with a decrease in heart rate that is less than 10% of the pre-CSM heart rate; or 3) mixed type, fall in systolic blood pressure ≥ 50 mm Hg associated with bradycardia ≥ 40 beats/min. The provocation of syncope or presyncope was necessary to establish the diagnosis of CSH. A positive HUTT was defined as induction of presyncope or syncope associated with a systolic blood pressure ≤ 70 mm Hg and/or bradycardia, resembling the clinical presentation (8). A modified version of the European classification for tilt-induced vasovagal syncope was used (8,9). Type 1 or mixed response was characterized by concurrent hypotension, systolic blood pressure ≤ 70 mm Hg and ventricular rate ≥ 40 beats/min. Type 2a cardioinhibitory response was indicated by a fall in ventricular rate < 40 beats/min for more than 10 s or asystole lasting more than 3 s. Blood pressure falls before the heart rate falls. Type 2b cardioinhibitory response was an abrupt fall in ventricular rate to < 40 beats/min for more than 10 s.

**Abbreviations and Acronyms**

CSH = carotid sinus hypersensitivity  
CSM = carotid sinus massage  
ECG = electrocardiogram  
HUTT = head-up tilt test  
VT = ventricular tachycardia  
VF = ventricular fibrillation

$\text{slope of the linear regression line}$

$\text{fall in systolic blood pressure}$

$\text{ventricular rate}$

40 beats/min for more than 10 s or asystole lasting more than 3 s. Blood pressure falls before the heart rate falls. Type 2b cardioinhibitory response was an abrupt fall in ventricular rate to < 40 beats/min for more than 10 s.
Table 1. Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>M/F</th>
<th>CAD</th>
<th>HBP</th>
<th>CVD</th>
<th>HCT</th>
<th>CS</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSH [+]</td>
<td>65 ± 12</td>
<td>38/10</td>
<td>15 (31%)</td>
<td>22 (45%)</td>
<td>5 (10%)</td>
<td>15 (31%)</td>
<td>4 (8.3%)</td>
<td>13 (27%)</td>
</tr>
<tr>
<td>CSH [-]</td>
<td>60 ± 11</td>
<td>20/12</td>
<td>8 (25%)</td>
<td>12 (38%)</td>
<td>3 (9%)</td>
<td>8 (25%)</td>
<td>2 (6.3%)</td>
<td>3 (9.3%)</td>
</tr>
<tr>
<td>Control</td>
<td>65 ± 14</td>
<td>25/5</td>
<td>3 (10%)</td>
<td>8 (26%)</td>
<td>0</td>
<td>5 (16%)</td>
<td>NA</td>
<td>2 (6.6%)</td>
</tr>
<tr>
<td>SNCSCH</td>
<td>59 ± 12</td>
<td>9/7</td>
<td>3 (16%)</td>
<td>6 (38%)</td>
<td>15 (94%)</td>
<td>3 (19%)</td>
<td>NA</td>
<td>1 (6.3%)</td>
</tr>
</tbody>
</table>

*p < 0.01.

CAD = coronary artery disease; CS = carotid sinus hypersensitivity; CVD = cardiovascular disease; HCT = hypercholesterolemia; HBP = high blood pressure; M/F = male/female; OH = orthostatic hypotension; SNCSCH = syncpe not related to carotid sinus hypersensitivity.

Table 2. Heart Rate and Blood Pressure Change After Pharmacologic, Mechanical and Orthostatic Stimulation of the Carotid Sinus

<table>
<thead>
<tr>
<th></th>
<th>HR B (beats/min)</th>
<th>ΔHR NT (beats/min)</th>
<th>ΔHR PH (beats/min)</th>
<th>ΔHR 0° (beats/min)</th>
<th>ΔHR 60° (beats/min)</th>
<th>ΔSBP 0° (mm Hg)</th>
<th>ΔSBP 60° (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSH [+]</td>
<td>70 ± 6</td>
<td>13 ± 8*</td>
<td>13 ± 8*</td>
<td>15 ± 9</td>
<td>28 ± 5*</td>
<td>18 ± 8</td>
<td>56 ± 24*</td>
</tr>
<tr>
<td>CSH [-]</td>
<td>72 ± 5</td>
<td>24 ± 10</td>
<td>21 ± 4</td>
<td>10 ± 8</td>
<td>10 ± 4</td>
<td>10 ± 6</td>
<td>14 ± 10</td>
</tr>
<tr>
<td>Control</td>
<td>71 ± 8</td>
<td>28 ± 8</td>
<td>22 ± 6</td>
<td>12 ± 10</td>
<td>11 ± 5</td>
<td>10 ± 6</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>SNCSCH</td>
<td>69 ± 9</td>
<td>11 ± 6*</td>
<td>9 ± 3*</td>
<td>13 ± 9</td>
<td>12 ± 6</td>
<td>12 ± 8</td>
<td>15 ± 9</td>
</tr>
</tbody>
</table>

*p < 0.001 CSH [-] versus control group, and CSH [-] versus SNCSCH group.

B = baseline; CSH = carotid sinus hypersensitivity; ΔHR = change in heart rate; NT = nitroprusside; PH = phenylephrine; ΔSBP = change in systolic blood pressure; SNCSCH = syncpe not related to carotid sinus hypersensitivity.
control group (Fig. 1; p < 0.001 for CSH vs. unexplained syncope and control groups). Similarly, a marked reduction in baroreflex slope was noted in the syncope unrelated to CSH group 1.5 ± 0.8 ms/mm Hg (p < 0.001 vs. unexplained syncope and control groups, p = 0.05 vs. CSH).

Nitroprusside baroreflex slopes were as follows: 2.8 ± 1.39 ms/mm Hg CSH group compared with 9.0 ± 1.7 ms/mm Hg, 12.2 ± 3.2 ms/mm Hg, 1.8 ± 0.95 ms/mm Hg in the unexplained syncope, control and syncope unrelated to CSH groups, respectively (p < 0.001 vs. control and unexplained syncope group, p < 0.05 vs. unrelated CSH syncope group). Syncope was not provoked during pharmacological stimulation of the carotid sinus in any patient.

Mechanical and orthostatic stimulation. The heart rate and blood pressure responses during CSM both in the supine position and upright 60° positions are shown in Table 2. Carotid sinus massage in the supine position was associated with a maximum change in heart rate of 15 ± 9 beats/min in the CSH group compared with 10 ± 8 beats/min, 12 ± 10 beats/min and 13 ± 9 beats/min in the unexplained syncope, control and syncope unrelated to CSH groups, respectively (p > 0.05). The maximum change in systolic blood pressure was 18 ± 8 mm Hg in the CSH group compared with 10 ± 6 mm Hg, 10 ± 4 mm Hg and 12 ± 8 mm Hg, in the unexplained syncope, control and syncope unrelated to CSH groups, respectively (p = 0.55 for CSH vs. other groups). In contrast, the maximum change in heart rate elicited by CSM in the 60° upright position were: 28 ± 5 beats/min in the CSH group compared with 10 ± 4 beats/min, 11 ± 5 beats/min and 12 ± 6 beats/min in the unexplained syncope, control and syncope unrelated to CSH groups, respectively (p < 0.001 for CSH vs. other groups). The maximum changes in systolic blood pressure during 60° CSM were: 56 ± 24 mm Hg in the CSH group, 14 ± 10 mm Hg, 12 ± 8 mm Hg and 15 ± 9 mm Hg in the unexplained syncope, control and syncope unrelated to CSH groups, respectively (p < 0.001 for CSH vs. other groups).

Carotid sinus hypersensitivity was elicited by CSM in the supine position in seven (8.7%) patients with unexplained syncope, two (6.6%) controls and in one (6.3%) patient with syncope unrelated to CSH. The response was significantly improved by CSM at 60° in 48 (60%) patients with unexplained syncope, compared with two (6.6%) controls and one (6.3%) patient in the syncope unrelated to CSH group. The response provoked by CSM was mixed in 25 (52%) patients, vasodepressor in 14 (29%) patients and cardioinhibitory in the remaining 9 (18%) patients with asystole ranging between 3 and 10 s. A mixed response was provoked in the two patients from the control group as well as the single patient from the unrelated CSH syncope group. The type of response elicited by CSM in the supine position was reproduced in all seven patients in the 60° upright position; however, the severity of the response was enhanced by orthostatic stress (Figs. 2 and 3). CSH was predominantly elicited by right CSM in 34 (70%) patients and by left CSM in the remaining 14 (30%) patients. A cardioinhibitory response was provoked in 6 (12.5%) patients during right CSM, and in 3 (6%) patients during left CSM, and a mixed response in 14 (29%) and 11 (23%) patients, respectively. A vasodepressor response was elicited in 8 (16%), and 6 (12.5%) patients, respectively. The effectiveness of CSM to detect CSH in the supine position was only 8.7% compared with 60% when CSM was performed during upright tilt. This provided an increase in diagnostic accuracy from 31% to 69%. Similarly, the positive predictive value was improved from 77% to 96% when CSM was performed during orthostatic stress. Head-up tilt test was positive in 12 (25%) patients with CSH, 2 (6.6%) controls and in none of the patients in which syncope remained unexplained after testing. A positive HUTT was also induced in one of the patients with syncope unrelated to CSH. The mean time of onset of syncope or presyncope was 12 ± 4.8 min, and the response elicited was type 1 (mixed) in four (33%) patients and type 3 (vasodepressor) in the remaining eight (67%) patients. The response provoked during HUTT was concordant with the response provoked by CSM in 9 of 12 (75%) patients.

DISCUSSION

The frequency of CSH as a cause of recurrent unexplained syncope varies between 2% and 14% and may be related to the routine assessment of CSM in patients referred for syncope evaluation (2,10,11). Similarly, standardization of the methodology of CSM has not been clearly established,
nor has the reproducibility of this test. The diagnostic value of routine and systematic stimulation of the carotid sinus by mechanical, pharmacological and orthostatic stress in patients with unexplained syncope has not been appropriately assessed.

CSM during orthostatic stress. The major finding of the present study was the significant increase in the diagnostic yield of CSM when applied in the upright position. Carotid sinus massage in the supine position identified only 7 (8.7%) patients with CSH. In contrast, the routine assessment of
CSM in the 60° upright position further identified CSH in 48 (60%) patients, increasing the diagnostic yield of CSM by 52%. Additionally, the unrelated CSH syncope group had the same rate of abnormal responses as the control group (6.3%). These findings are in agreement with a recent report from McIntosh et al. (12) in which more than half of a series of elderly patients with recurrent syncope would have been missed if CSM had not been performed in the upright position, failing to identify CSH as the cause of the syncopal episodes. The inclusion of a control group and a group of patients with syncope not related to CSH permitted us to calculate the sensitivity, specificity and predictive values of CSM during orthostatic stress. It should be noted that due to the lack of a true gold standard, the sensitivity reported herein simply reflects the number of subjects in which CSH was documented. Nonetheless, it is clear that the diagnostic accuracy of CSM was markedly increased from 31% in the supine position to 69% in the upright position. Similarly, the positive predictive value was enhanced from 77% to 96% with excellent specificity (93%). These findings support the recommendation to perform CSM in both the supine and upright positions in older patients with recurrent unexplained syncope.

Mechanisms. The mechanism underlying the additive effects of CSM and orthostatic stress are unclear. However, neurocardiogenic syncope triggered by both HUTT and CSH may share a common reflex pathway (13,14). This is suggested by our observation that CSH and HUTT elicited neurocardiogenic reflex responses in 25% of patients. Mechanical stimulation of the carotid sinus in combination with orthostatic stress may sensitize both arterial and

Figure 3. Continuous ECG and blood pressure recording during right CSM (60°) in the same patient as in Figure 2. Right carotid massage elicited abrupt asystole lasting 5.1 s associated with syncope.
cardiopulmonary baroreceptors and contribute to the activation of the neurocardiogenic reflex. In the current study, HUTT provoked syncope in 25% of patients with unexplained syncope and CSH. This finding further supports the hypothesis that CSH and neurocardiogenic syncope may be part of the spectrum of the same neurocardiogenic reflex and may share common pathophysiologic processes. The incidence of HUTT-induced neurocardiogenic syncope is low compared with previous reports. However, this finding may be related to the characteristics of the group assessed, which was primarily older aged patients with fewer number of syncopal episodes in the six months preceding evaluation and which included patients with dizziness and presyncope.

Another important finding is the significant reduction in the baroreceptor reflex sensitivity slope we documented in patients with syncope and CSH. These findings may be related to an impaired baroreceptor adaptation to orthostatic stress that may lead to inappropriate modulation of muscle sympathetic nerve activity and further withdrawal of sympathetic activity at the time of hypotension, potentiating the development of syncope. Simultaneous recording of muscle sympathetic activity at the time of syncope provoked by CSM has documented abrupt sympathetic withdrawal at the onset of hypotension (14). Further insight into this mechanism may be provided by our finding of a reduced response in blood pressure to bolus injections of nitroprusside, suggesting impaired efferent sympathetic baroreflex traffic. O’Mahony (13) has recently proposed that medullary α-2 receptor upregulation may be related to the pathophysiology of CSH. Our findings neither confirm nor disprove this hypothesis. However, the decreased baroreceptor response to vasoactive agents may be related to these findings. A marked reduction in baroreflex gain (<2 ms/mm Hg) was also noted in the group of patients with syncope not related to CSH. This is no surprise, given the fact that the majority of subjects in this group had documented VT/VF associated with Chagas cardiomyopathy. We have previously reported a significant reduction in baroreflex gain in sudden cardiac death survivors with Chagas cardiomyopathy (15). Other investigators have previously documented a high risk for induction of VT/VF in postinfarction patients with reduced baroreflex sensitivity <3 ms/mm Hg (16–18). The pathophysiologic role of reduced baroreflex gain in the CSH group and the group with syncope not related to CSH remains unclear. However, Landolina et al. (19) have recently suggested that the hemodynamic tolerance to VT may be related to impaired cardiovagal reflexes; namely, markedly reduced baroreflex gain (<2 ms/mm Hg) was associated with poorer hemodynamic tolerance of VT. Impaired baroreflex gain may simply be a harbinger of orthostatic intolerance in both CSH and VT/VF.

Additionally, our group and other investigators have recently documented a significant reduction in baroreceptor gain in patients with neurocardiogenic syncope provoked by HUTT (20–22), further supporting these findings. However, Morley et al. (23) and Dehn et al. (24) have reported opposite findings, namely increased baroreceptor gain in patients with CSH. The nature of this discrepancy is unclear and may be attributed to differences in the methodology used for assessment of baroreceptor reflex sensitivity.

Syncope or presyncope was not provoked in any of our patients during pharmacologic stimulation. This finding is consistent with Morley et al. (23), who did not report inducing syncope or presyncope in any of their patients during phenylephrine infusion. Similarly, Dehn et al. (24) did not report the induction of syncope of presyncope during carotid stimulation using a neck chamber. The explanation for this finding is unclear; however, it is possible that both pharmacologic and mechanical stimulation with the neck chamber produce a less intense stimulation of the carotid sinus than direct external pressure. Similarly, in our study and in previously reported studies (23,24), pharmacologic and mechanical stimulation with the neck chamber have been performed exclusively in the supine position. Based on our findings, it is possible that both pharmacologic and mechanical stimulation with the neck chamber during orthostatic stress may increase the diagnostic yield of these methods.

The response obtained during CSM was primarily mixed in 52%, vasodepressor in 29% and only 18% presenting as cardioinhibitory CSH. These findings contrast with previous reports in which the cardioinhibitory response was documented in up to 80% of patients (2–5). Most previous studies have reported that vasodepressor responses account for only 5% to 10% of CSH cases (1–5,25,26). The explanation for this difference may be due to the lack of continuous noninvasive blood pressure recordings in the earlier reports as well as absence of simultaneous CSM during orthostatic stress (12). Gaggioli et al. (27) have recently highlighted the importance of beat-to-beat blood pressure measurements during CSM and reported a vasodepressor response in 84% of patients with CSH. We observed a vasodepressor response in 29% of patients; however, we did not perform CSM after administration of atropine, possibly underestimating the incidence of the vasodepressor response.

**Study limitations.** The lack of a gold standard method for the diagnosis of syncope limits the findings of CSH as the exclusive cause of syncope. Electrophysiologic evaluation was not performed in all subjects, rendering the possibility that other potential causes of syncope were not excluded. Elderly patients are more likely to have multiple causes of syncope coexisting. Similarly, CSM was not repeated after the administration of atropine, possibly underestimating the frequency of the vasodepressor response. Nonetheless, it is unlikely that these limitations invalidate our findings.

**Conclusions.** CSH was documented in 68% of patients with unexplained syncope, 8% in the supine position and 60% in the upright position, referred for assessment of
unexplained syncope or presyncope. Combined orthostatic stress and mechanical stimulation of the carotid sinus markedly increased the diagnostic yield of CSM. Decreased baroreceptor gain was documented in patients with CSH and may play a role in the pathophysiology of CSH.

Reprint requests and correspondence: Dr. Carlos A. Morillo, Department of Cardiology and Cardiovascular Sciences, Fundación Cardiovascular del Oriente, Colombiano Instituto del Corazón, Calle 155A, No. 23-58, Urbanización El Bosque, Florida-blanca, Santander, Colombia.

REFERENCES