Original article

Incidence and Prognostic Value of Ischemic Heart Disease in High Risk Cardiovascular Asymptomatic Diabetic Patients Detected by Gated Myocardial Perfusion SPECT Study

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ABSTRACT

Aim: To determine the clinical utility of the gated myocardial perfusion SPECT to detect silent ischemia in asymptomatic diabetic patients without previous coronary events and to evaluate the prognosis of this population.

Material and methods: A retrospective study of 56 asymptomatic diabetics referred for a gated myocardial perfusion SPECT for the diagnosis of ischemic disease was performed. The criteria for ischemia were: mild SDS < 4, moderate SDS 4–8, severe SDS > 8. A multivariable statistical analysis was carried out to identify possible predictive variables of an abnormal SPECT. The cardiovascular events were recorded up to December-2010.

Results: A high proportion of the 56 patients had an abnormal perfusion study (46.4%), there being moderate–severe ischemia in 10.7%, necrosis with ischemia in 5.4% and necrosis in 7.1%. We found no statistical differences in the type of stress used (treadmill or dipyridamole). The patients had a high combination of cardiovascular risk factors. In the multivariate analysis, diabetic nephropathy was the only factor related to an abnormal SPECT (p = 0.043). The events recorded in the follow-up were: 2 early revascularizations, 5 cardiology admissions, 10 non-cardiac related deaths. The existence of ischemia in the SPECT was significantly related to the appearance of cardiovascular events (p < 0.05).

Conclusion: A gated myocardial perfusion SPECT in asymptomatic diabetics with high combination of cardiovascular risk factors detects silent ischemia in a significant proportion and this seems to be related to future coronary events. Diabetic nephropathy implies a greater likelihood of abnormal studies. However, the screening criteria in this population still need to be established for better performance and lower cost.

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PALABRAS CLAVE:
Diabetes mellitus
Silente ischemic heart disease
Gated SPECT
Myocardial perfusion imaging

RESUMEN

Objetivo: Determinar la utilidad clínica de la gated-SPECT de perfusión miocárdica en la detección de isquemia silente en pacientes diabéticos sin síntomas ni eventos cardiovasculares previos y evaluar implicaciones pronósticas.

Material y métodos: Estudio retrospectivo de 56 pacientes diabéticos asintomáticos tras una gated-SPECT de perfusión miocárdica para diagnóstico de enfermedad isquémica. El criterio de isquemia fue: ligera SDS < 4, moderada SDS de 4 a 8, severa SDS > 8. Se realizó un análisis estadístico multivariante para identificar variables predictores de un estudio anormal y se registraron hasta diciembre de 2010 los eventos cardiovasculares.

Resultados: Una alta proporción de los 56 pacientes presentó un estudio de perfusión anormal (46.4%), existiendo isquemia moderada–severa en el 10.7%, necrosis con isquemia en el 5.4% y necrosis en el 7.1%. No encontramos diferencias en cuanto al tipo de esfuerzo (tapiz rodante o dipiridamol). Existió una alta combinación de factores de riesgo cardiovascular. En el análisis multivariante, la nefropatía diabética fue la única que se relacionó con un SPECT anormal (p = 0.043). En el seguimiento, los eventos fueron: 2 revascularizaciones precoce, 5 ingresos en cardiología, 10 muertes de no origen cardiaco. La existencia de isquemia en la SPECT se relacionó de forma significativa con la aparición de eventos cardiovasculares (p < 0.05).

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Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by elevated levels of baseline glycemia because of inadequate insulin secretion, resistance in the action of the insulin or both mechanisms. Chronic hyperglycemia of diabetes is associated to long-term damage, with dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels.\(^1\)

The American Diabetes Association (ADA) distinguishes two varieties according to the pathophysiology of the disease: Type 1 DM, that accounts for 5–10%, associated to young persons and related to limited release of insulin caused by destruction of pancreatic \(\beta\) isolates. Type 2 DM, the remaining 90–95%, is associated to age and insulin resistance. This type 2 is undergoing great expansion due to the tendency to sedentary life and to the inversion of the demographic pyramids as a consequence of population aging. The approximate number of diabetic persons in the world is 170 million and it is expected that this number will be doubled in 2030. In Spain, the estimated prevalence of DM is approximately 6.5% of the population. Therefore, we are faced with one of the important social-healthcare and social economical problems of recent years worldwide.\(^2,3\)

The diagnostic criteria of diabetes are undergoing continuous change and renewal. In the last review of the ADA in January 2010, the upper threshold of fasting glycemia has been reduced from \(>140\) mg/dl to \(>126\) mg/dl. Glycemia has been maintained at \(\geq 200\) mg/dl after stimulus of oral glucose overload and glycosylated hemoglobin (HbA\(_1c\)), a reflection of the glucose situation of the last 2–3 months of life of the patient, is HbA\(_1c\) \(\geq 6.5\%\).

Within the cardiovascular setting, DM is one of the most relevant risk factors of coronary artery disease. This is increasing in prevalence and frequently occurs without symptoms. In fact, diabetic patients without previous infarction or cardiovascular disease have the same prognosis as non-diabetes patients, but with infarction or cardiovascular disease.\(^4\) Macrovascular involvement, especially heart disease, is the principal cause of morbidity–mortality in this group of diabetic patients, since untreated DM or poorly controlled DM is associated to an increase of cardiovascular risk that is 2–4 times greater compared with well-controlled diabetic patients and the rest of the general population.\(^5,6\) According to the results of a meta-analysis of prospective studies of a cohort conducted in type 2 DM patients, it was concluded that cardiovascular risk increased 18% (RR: 1.18 [95% CI: 1.10–1.26]) for every 1% increase in HbA\(_1c\).\(^7\)

These complications, attributable to the arteriosclerosis, account for 75% of hospitalizations due to diabetic complications and for 70–80% of the causes of death among diabetic patients.

In diabetic patients with cardiovascular symptoms, there is an international consensus to recommend the combination of stress studies with electrocardiographic control and imaging tests (SPECT, ultrasonography, MR, CT scan) as non-invasive diagnostic method. Therefore, if the tests performed make it possible to classify the patients as low risk, clinical management must be based on an aggressive medical treatment of control of risk factors, changing to invasive treatment if the symptoms persist or increase. The period of time of guarantee of a normal non-invasive test in these symptomatic patients is considered to be 2 years, so that it is recommended that routine follow-up studies be performed every 2 years.\(^8,9\)

However, silent ischemia is a frequent condition of diabetic patients, which is why evaluation of the performance of systematic screenings in asymptomatic patients with high cardiovascular risk is being done.\(^10,11\) Both the multicenter DIAD study, in its different updates\(^12–16\) and the more recent DYNAMIT,\(^17\) have questioned the utility of this type of screening. Their results reflect that although the prevalence of silent ischemia in the SPECT is about 22–35%, there are no differences regarding cardiovascular events among patients who undergo aggressive treatment or do not undergo it. It is true that in both studies, there was a high relation of mild ischemia and low number of patients with moderate or severe ischemia. This partially justifies the lack of differences with the baseline group of normality. In any case, the diagnostic algorithm in these patients continues to lack consensus. As in low risk symptomatic patients, the general recommendation in these asymptomatic patients is adequate control of the risk factors and of the diabetes.

This study has aimed to determine the clinical utility of the myocardial perfusion gated-SPECT in the early detection of perfusion alterations of diabetic patients who have not had previous symptoms or cardiovascular events and to evaluate its prognostic implications.

Material and methods

Population

From 2001 to 2009, 463 diabetic patients were seen in the nuclear cardiology unit of our hospital for a myocardial perfusion gated-SPECT for diagnostic purposes. For patient inclusion in the study, the following were considered: non-presence of cardiovascular symptoms or electrocardiographic alterations suggestive of previous ischemia or necrosis (alterations of the ST segment or Q waves). All those diabetic patients with a previous history of ischemic heart disease or who had symptoms prior to the performance of the test were excluded.

A total of 56 cases who did not have cardiovascular symptoms and who had not been previously diagnosed of ischemic heart disease were enrolled. Of these, 46 were male and 10 women, with a mean age of 65.89 years.

Gated-SPECT

All of the subjects enrolled underwent a myocardial perfusion gated-SPECT study, using the one day short protocol, which included a first stress phase and a second rest phase. In those cases in which the stress study had perfusion images, ventricular values and normal contractibility, no rest study was performed. Preparation of the patient means fasting for at least 3 h prior to the test, including the withdrawal of the medication that could alter its result.\(^18,19\) All the patients signed the informed consent according the ethics committee criterion of our hospital.

Depending on the medical criteria and circumstances of the patients (age, previous mobility, tolerance to exercise, etc.), two types of stress tests were performed: an exercise test on treadmill, according to the Bruce protocol, or a pharmacological stimulation test with diprydiamole at high dose (0.84 mg/kg) in 6 min.

The radiotracer used was \(^{99m}\)Tc-Tetrofosmin (Myoview\(^8\)), with a dose of 296–370 MBq in the stress test and 740–925 MBq for...
Table 1
Characteristics of the patients: comparison between patients with normal and abnormal myocardial perfusion studies.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All the patients (n=56)</th>
<th>Abnormal study (n=26)</th>
<th>Normal study (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.150</td>
</tr>
<tr>
<td>Men</td>
<td>40 (71.4)</td>
<td>21 (80.8)</td>
<td>19 (63.3)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>16 (28.6)</td>
<td>5 (19.2)</td>
<td>11 (36.7)</td>
<td></td>
</tr>
<tr>
<td>Age (years) &lt;60</td>
<td>11 (19.6)</td>
<td>3 (11.5)</td>
<td>8 (26.7)</td>
<td>0.050</td>
</tr>
<tr>
<td>Age (years) ≥60</td>
<td>45 (81.4)</td>
<td>23 (88.5)</td>
<td>22 (73.3)</td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI)</td>
<td></td>
<td></td>
<td></td>
<td>0.458</td>
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<tr>
<td>Low weight (&lt;18.5)</td>
<td>10 (17.9)</td>
<td>4 (15.4)</td>
<td>6 (20)</td>
<td></td>
</tr>
<tr>
<td>Normal (18.6–24.9)</td>
<td>36 (64.3)</td>
<td>15 (57.7)</td>
<td>21 (70)</td>
<td></td>
</tr>
<tr>
<td>Overweight (25–29.9)</td>
<td>8 (14.3)</td>
<td>5 (19.2)</td>
<td>3 (10)</td>
<td></td>
</tr>
<tr>
<td>Obesity (≥30)</td>
<td>2 (3.6)</td>
<td>2 (6.9)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Smokes</td>
<td></td>
<td></td>
<td></td>
<td>0.747</td>
</tr>
<tr>
<td>Current</td>
<td>17 (30.4)</td>
<td>9 (34.6)</td>
<td>8 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>8 (14.3)</td>
<td>4 (15.4)</td>
<td>4 (13.3)</td>
<td></td>
</tr>
<tr>
<td>AHT</td>
<td>52 (92.9)</td>
<td>26 (100)</td>
<td>26 (86.7)</td>
<td>0.043*</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>28 (50)</td>
<td>16 (61.5)</td>
<td>12 (40)</td>
<td>0.124</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>24 (42.9)</td>
<td>9 (34.6)</td>
<td>15 (50)</td>
<td>0.246</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>22 (39.3)</td>
<td>7 (26.9)</td>
<td>15 (50)</td>
<td>0.048*</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>6 (10.7)</td>
<td>3 (11.5)</td>
<td>3 (10)</td>
<td>0.853</td>
</tr>
<tr>
<td>Peripheral arteriopathy</td>
<td>8 (14.3)</td>
<td>4 (15.4)</td>
<td>4 (13.7)</td>
<td>0.827</td>
</tr>
<tr>
<td>HbA1c &gt; 6.5%</td>
<td>27 (48.2)</td>
<td>9 (34.6)</td>
<td>18 (60)</td>
<td>0.132</td>
</tr>
<tr>
<td>Type of stress</td>
<td></td>
<td></td>
<td></td>
<td>0.866</td>
</tr>
<tr>
<td>Stress (St)</td>
<td>28 (50)</td>
<td>12 (46.2)</td>
<td>16 (53.4)</td>
<td></td>
</tr>
<tr>
<td>Dipyrudamole (Dip)</td>
<td>26 (46.4)</td>
<td>13 (50)</td>
<td>13 (43.3)</td>
<td></td>
</tr>
<tr>
<td>St + Dip</td>
<td>2 (3.6)</td>
<td>1 (3.8)</td>
<td>1 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Clinical result</td>
<td></td>
<td></td>
<td></td>
<td>0.278</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>55 (98.2)</td>
<td>26 (96.2)</td>
<td>31 (100)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (1.8)</td>
<td>1 (3.8)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Electrical result</td>
<td></td>
<td></td>
<td></td>
<td>0.102</td>
</tr>
<tr>
<td>Negative</td>
<td>38 (67.9)</td>
<td>15 (57.7)</td>
<td>23 (76.7)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>8 (14.3)</td>
<td>3 (11.5)</td>
<td>5 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Not evaluable</td>
<td>10 (17.9)</td>
<td>8 (30.7)</td>
<td>2 (6.7)</td>
<td></td>
</tr>
<tr>
<td>Ejection-St fraction</td>
<td></td>
<td></td>
<td></td>
<td>0.253</td>
</tr>
<tr>
<td>EFst &lt; 40%</td>
<td>11 (19.6)</td>
<td>7 (26.9)</td>
<td>4 (13.3)</td>
<td></td>
</tr>
<tr>
<td>EFst &gt; 40%</td>
<td>45 (71.4)</td>
<td>19 (74.1)</td>
<td>26 (86.7)</td>
<td></td>
</tr>
<tr>
<td>Ejection-rest fraction (rest)</td>
<td></td>
<td></td>
<td></td>
<td>0.040*</td>
</tr>
<tr>
<td>EJrest &lt; 35%</td>
<td>12 (21.5)</td>
<td>8 (30.8)</td>
<td>4 (13.3)</td>
<td></td>
</tr>
<tr>
<td>EJrest &gt; 35%</td>
<td>44 (78.5)</td>
<td>18 (69.2)</td>
<td>26 (86.7)</td>
<td></td>
</tr>
</tbody>
</table>

* Significance value p < 0.05.

the subsequent rest study. Intravenous administration of the radiotracer was performed when theoretical maximum heart rate reached 85% in the cases of stimulation on the treadmill and after 3 min of the dipyridamole injection in the pharmacological stimulation. Those subjects who did not achieve the desired rate were stimulated with atropine. Acquisition of the images was performed approximately between 20 and 30 min after injection of the radiotracer for better evaluation of the possibility of stunned myocardium.

The equipment used was a Picker Axis Prism II dual detector gamma camera with an angle between heads of 102° with low energy and high resolution collimator. Semicircular orbit was made between right anterior oblique projection to 45° until left posterior oblique projection, completing a 180° orbit. A 64 × 64 matrix was used, energy window of 20% centered on the 140 keV and zoom 1882, acquiring a step & shoot mode, with 3 steps, therefore generating 34 projections with a time of 25 s per projection.

The myocardial perfusion study was performed in synchronization with the electrocardiogram. The number of images, both for the stress and rest study, was 8 images per cardiac cycle. Iterative reconstruction with a Low Pass, Order 5 filter and 0.30 frequency for the rest studies was used for processing the images. Cross-sectional short axis, horizontal long axis and vertical long axis tomography slices were obtained.

According to the standard division of the ASCN and AMA into 17 segments of the left ventricle, myocardial perfusion uptake values were collected, obtaining scores for stress, rest and differential (summed differential score—SDS). Polar map was obtained for coronary vessels and segments according to the QPS program and the calculation of the ejection fraction and ventricular volumes with the QGS quantitative program.

Myocardial ischemia criteria considered in accordance with the SDS were: no or mild ischemia if SDS < 4; moderate ischemia if 4–8 points and severe ischemia if greater than 8 points (SDS > 8). Thus, the studies were classified as normal, ischemia (mild, moderate, severe), necrosis and necrosis with ischemia.

Other variables collected in the study included: age of patient, arterial hypertension (AHT), dyslipidemia, obesity (according to the body mass index [BMI]), smoking habit, presence of retinopathy, nephropathy or peripheral arteriopathy.

According to the HbA1c levels, it was considered that there was good control of the DM when there was an HbA1c level of 6.5% and poor control with levels over 6.5% at the time of the study.

In the follow-up until December 2010 (mean = 5.7 years; range 1.2–9.8 years), the events recorded were: need for early revascularization, admission associated to ischemic related or non-related heart disease (arrhythmias, heart failure), death of cardiac origin and global mortality.
Table 2
Result of the myocardial perfusion SPECT.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30</td>
<td>53.6</td>
</tr>
<tr>
<td>Mild ischemia SDS &lt; 4</td>
<td>13</td>
<td>23.2</td>
</tr>
<tr>
<td>Moderate–ischemia SDS 4–8</td>
<td>4</td>
<td>7.1</td>
</tr>
<tr>
<td>Severe ischemia SDS &gt; 8</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>Necrosis ± ischemia</td>
<td>4</td>
<td>7.1</td>
</tr>
<tr>
<td>Necrosis</td>
<td>2</td>
<td>5.4</td>
</tr>
</tbody>
</table>

The statistical analysis was carried out with the SPSS program, version 15.0 for Windows with a statistical significance level of $p < 0.05$.

Results

Our sample was made up of 56 patients, 46 of whom were men (71.4%) and 10 women (28.6%), with mean age of 65.89 years. Six of the patients were diagnosed of type 1 DM (10.7%) and 50 of type 2 DM (89.3%).

These patients had high combination of coronary risk factors, reason why conducting a myocardial perfusion gated-SPECT was indicated, although some were a result of pre-operative or pre-transplant studies. Prevalence of coronary risk factors was: obesity or overweightness (BMI > 25) in 10 cases (17.9%); active smoker in 17 (30.4%) and ex-smoker in 8 (14.3%); AHT in 52 (92.9%); hypercholesterolemia in 28 (50%); peripheral arteriopathy in 8 (14.3%); neuropathy in 6 (10.3%); retinopathy in 24 (42.9%) and nephropathy in 22 (39.3%) (Table 1).

On the date of the study, we found that the percentage of HbA1c was greater than 6.5% (patients whose DM was poorly controlled) in 27 patients (48.2%).

The stress stimulus was performed on treadmill in 28 cases (50%), with dipyridamole in 26 cases (46.4%) and in combined stress-dipyridamole in 2 cases (3.6%). The test outcome was clinically positive in one case (1.8%) and electrophisically positive in 8 cases (14.3%). However, there were previous alterations in the electrocardiogram in 17.9% of the patients so that the electrical response could not be evaluated (BRI, overload of left ventricle due to AHT, use of drugs such as digoxin associated to atrial fibrillation, etc.).

The stress stimulus of the myocardial perfusion SPECT had a normal result in 30 (53.6%) of the patients. It was abnormal in 26 patients (46.4%), there being mild ischemia (SDS < 4) in 13 (23.2%), moderate (SDS = 4–8) in 4 (7.1%), severe ischemia (SDS > 8) in 2 (3.6%), necrosis in 4 (7.1%) and necrosis associated ischemia in 3 (5.4%) (Table 2).

In the follow-up of the 56 patients until December 2010 (mean: 5.7 years; range: 1.2–9.8), the events registered were: early revascularization after the SPECT in 2 patients (3.6%). Both cases had appearance of cardiovascular events (Fig. 1).

Discussion

DM is one of the major cardiovascular disease risk factors, coronary macrovascular involvement being the principal cause of morbidity–mortality in these patients. Thus, screening methods are being conducted to detect those patients who may suffer coronary arterial disease to establish early therapeutic measures and thus decrease the fatal cardiovascular events in this population.

The difficulty to detect coronary artery disease in diabetic patients is because it occurs asymptotically in a large percentage of patients. There is growing interest in evaluating the incidence of coronary artery disease in asymptomatic diabetic patients, using imaging techniques, among them the myocardial perfusion SPECT.

In the literature, there are several prospective studies that clearly show the utility of the myocardial perfusion SPECT in the detection of myocardial ischemia. Thus, in this population perfusion, alterations are expected in approximately 15–33%, there being moderate–severe grade ischemia in 6–8%.

Our sample includes a high rate of hypertensive patients, with predominance of type 2 DM and an elevated incidence of cardiovascular risk factors, within that to be expected in a population having long evolution of diabetes (neuropathy, retinopathy, nephropathy), although it has a low proportion of obese patients (19.9%). We have found a high incidence of abnormal SPECT (46%), moderate–severe ischemia of 9% having been observed, similar to other studies. However, in our casuistics there is an important proportion of patients with necrosis (5.4%) or necrosis with associated ischemia (7.1%). These patients have basal alterations on their electrocardiogram that do not make it possible to identify them as being susceptible to ischemic type disease. Therefore, they comply with the inclusion criteria in this type of retrospective analysis.

A univariate and multivariate study was performed in order to identify possible variables were risk factors that could orient a profitable screening algorithm of asymptomatic diabetic patients. The existence of nephropathy was the only variable in the multivariate analysis of regression that had statistical significance in relation to the abnormal SPECT result. There is no clear relationship between the existence of conventional risk factors and ischemia on the SPECT in the different existing publications. Thus, in a prospective study of Anand et al., no cardiovascular factor served to predict ischemia.

In the DIAD study, this factor was diabetic neuropathy; in that of Schoelte et al., it was being a smoker, time of evolution of the diabetes and the cholesterol/HDL ratio, and recently Hernández et al. proposed retinopathy as a risk factor to be considered in this
Figure 1. Myocardial perfusion SPECT study with post-stress and rest images, and polar map images. They correspond to a 68 year old woman, with AHT, type 2 DM, with signs of ventricular hypertrophy in the baseline ECG. This is the only case that is clinically positive but not limiting. Ischemia was observed in anterior and lateral fact and early revascularization was performed in descending anterior and circumflex arteries.

In spite of the limitations of our study (retrospective, with wide margin of patient screening and very high rate of associated cardiovascular risk factors), the existence of ischemia in the perfusion SPECT was statistically related to the events occurring in the cardiovascular sphere, both in early revascularization and in ischemic-origin hospital admissions. When this finding is applied to our clinical practice, an asymptomatic, hypertense diabetic patient with nephropathy, referred for a diagnostic study of ischemic heart disease, will need intensive treatment if there is a positive SPECT for ischemia, given the relation to a possible cardiovascular event in 5–7 months.

However, given the limitations indicated, to reach a grade of generality, the prospective study DIAD whose results have been published in recent years,12–16 as well as the more recent DYNAMIT study, should be considered. We believe that beginning with these studies, the use of the myocardial perfusion SPECT screening method in asymptomatic diabetic patients has made it possible to classify them more effectively into high and low risk. However, given that this fact does not affect future events, the use of diagnostic methods in diabetic patients must be delayed until the appearance of symptoms given the high financial cost and low rate of expected events. Establishing and knowing what the risk factors are in diabetes that would make it possible to adequately select the study population continues to be necessary.

Conclusion

The myocardial perfusion SPECT in asymptomatic diabetics with high association of risk factors detects the existence of silent ischemia. This seems to be related with future cardiovascular events. Diabetic nephropathy suggests greater likelihood of abnormal studies. However, it is necessary to establish the screening criteria for better yield and lower financial cost.
Conflict of interests
The authors declare they have no conflict of interests.

Acknowledgements
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References