Cardiac Autonomic Neuropathy in the Diabetic Patient: Does ^{123}I-MIBG Imaging Have a Role to Play in Early Diagnosis?

Lynda A. Scott, DipRad (D)(NM)(RT); and Peter L. Kench, BAppSc (Hons)

School of Medical Radiation Sciences, University of Sydney, New South Wales, Australia

Objectives: In diabetes, extended adrenergic receptor stimulation with hyperglycemia and insulin deficiency is associated with cardiac autonomic dysfunction. Clinically evident diabetic cardiac autonomic neuropathy (CAN) is associated with a poor prognosis. Research studies indicate that autonomic function tests, which are traditionally used to diagnose diabetic CAN, are less sensitive than ^{123}I-metaiodobenzylguanidine (MIBG) imaging, particularly in the early stages of the disease. This established imaging technique makes use of the noradrenaline analog MIBG, which is radiolabeled with ^{123}I to assess the noradrenaline uptake-1 mechanism of the sympathetic nervous system. Although scintigraphic studies indicate that long-standing cardiac autonomic dysfunction is permanent, some authors have shown partial reversibility with early metabolic intervention. ^{123}I-MIBG imaging could therefore have an important clinical role to play in the early diagnosis and treatment monitoring of diabetic CAN.

Methods: A PubMed/MEDLINE Internet search was performed using *MIBG, diabetes,* and *cardiac autonomic neuropathy* as key words.

Conclusion: The general expense of ^{123}I-MIBG imaging, together with the lack of commercial availability of this radiopharmaceutical in the United States, has limited the clinical use of this technique. As such, the clinical role of ^{123}I-MIBG imaging in the early diagnosis of diabetic CAN has yet to be validated and defined in most regions of the world, and further study is required.

Key Words: diabetes; cardiac autonomic neuropathy; ^{123}I-metaiodobenzylguanidine; MIBG

J Nucl Med Technol 2004; 32:66–71

Cardiac autonomic neuropathy (CAN) is a common complication of diabetes and is associated with resting tachycardia, postural hypotension, painless myocardial ischemia or infarction, arrhythmias, and sudden cardiac death (*1*). It is believed that the progression of CAN can be halted and perhaps even reversed with early metabolic intervention (*2–4*). In clinical practice, CAN is most commonly assessed by indirect autonomic function tests, which are believed to underestimate early autonomic dysfunction (*3,5*). ^{123}I-Metaiodobenzylguanidine (MIBG) imaging is a direct, noninvasive in vivo method of assessing autonomic function, allows for objective semiquantitative assessment (*5*), and has been shown to detect diabetic CAN even in the early stages of the disease (*6*). This technique has been widely used in research to assess diabetic CAN but, with the exception of Japan, has had limited clinical use.

This paper reviews the relationship between diabetes and CAN to establish the importance of early diagnosis. ^{123}I-MIBG imaging in diabetic CAN is also reviewed, highlighting the advantages and disadvantages in comparison with other diagnostic techniques. Obstacles that have inhibited the widespread clinical implementation of this technique are discussed to possibly explain the disparity between clinical use in Japan, Europe, and the United States, and areas of possible future study are identified. Publications were identified by a PubMed/MEDLINE Internet search using *MIBG, diabetes,* and *cardiac autonomic neuropathy* as key words.

CARDIAC SYMPATHETIC INNERVATION

The autonomic nervous system, which includes the parasympathetic and sympathetic systems, plays an important role in the regulation of myocardial function, heart rate, and myocardial blood flow (*7*). The sympathetic system innervates the myocardium via sympathetic nerve fibers that traverse the subendocardium along the path of the coronary vessels, from the base to the apex of the heart (*6,8*). Noradrenaline (norepinephrine) is the main neurotransmitter of

the sympathetic nervous system, and activation of this system or an increase in catecholamine level results in an increased heart rate (chronotropic effect), enhanced contractility (inotropic effect), and improved atrioventricular conduction (dromotropic effect) in the normal heart (6,9). Noradrenaline is synthesized from tyrosine in adrenergic neurons and is stored in membrane-bound adrenergic granules (10). During neurotransmission, the granule contents are discharged by exocytosis (10). Noradrenaline binds to postsynaptic β_1-receptors located mainly in the synaptic cleft (6). The neuronal uptake-1 mechanism is the sodium- and energy-dependent homeostatic system responsible for reuptake of noradrenaline (6). Extended increased stimulation of cardiac adrenergic receptors results in desensitization and downregulation of the receptors, as well as increased receptor degradation and decreased receptor synthesis (6,9).

PATHOPHYSIOLOGY OF CAN IN DIABETES

The sympathetic nervous system is stimulated in the early stages of diabetes (6,11), and extended exposure of the adrenergic receptors to increased catecholamine levels, together with hyperglycemia and insulin deficiency, is believed to cause diabetic CAN (6). The advent of direct imaging techniques, including ^{123}I-MIBG scintigraphy and PET neurotransmission studies, has allowed researchers to explore the natural progression of diabetic CAN.

^{123}I-MIBG AND THE SYMPATHETIC NERVOUS SYSTEM

MIBG is a noradrenaline analog that is produced through a modification of guanethidine (9) and is labeled with ^{123}I to allow for imaging. ^{123}I-MIBG presynaptic neuronal uptake is predominantly through the uptake-1 mechanism (Fig. 1) (6,11). Although studies have shown that nonneuronal uptake (uptake-2 mechanism) of ^{123}I-MIBG by the myocardium is not significant (12), this uptake mechanism has been shown to be significant at high plasma ^{123}I-MIBG concen-
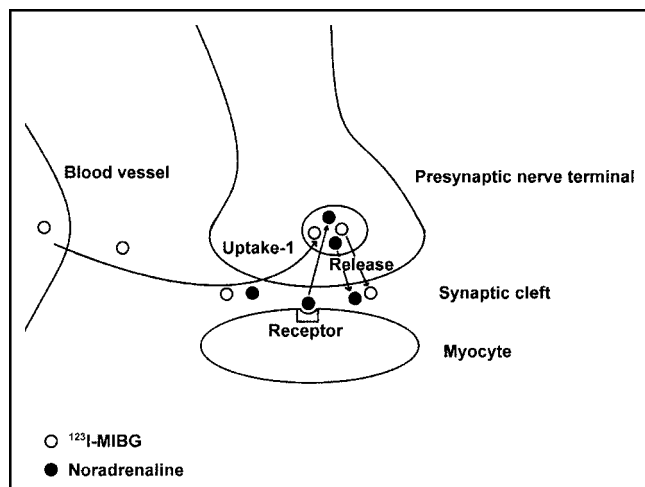

FIGURE 1. Schematic representation of the ^{123}I-MIBG uptake mechanism.

trations, likely because of passive diffusion (6). However, the uptake-1 mechanism predominates in ^{123}I-MIBG imaging because of the small quantity of ^{123}I-MIBG injected (6). The normal heart has dense sympathetic innervation and shows good uptake of ^{123}I-MIBG (6). ^{123}I-MIBG does not undergo intracellular metabolism (7). However, studies have shown that ^{123}I-MIBG follows the same active uptake, storage, and release pathways as does noradrenaline. Thus, quantitative ^{123}I-MIBG imaging is believed to allow assessment of global and regional sympathetic integrity by measurement of uptake, and assessment of function by measurement of washout (10,13).

PHARMACOLOGIC INTERACTIONS WITH MIBG

Studies have shown that some medications and physiologic states interfere with the biodistribution of ^{123}I-MIBG (10). Cocaine and the tricyclic antidepressant desmethylimipramine specifically inhibit the type-1 catecholamine reuptake system and have been shown to reduce ^{123}I-MIBG uptake (10,14). Although conventional α- or β-blocking medications do not affect ^{123}I-MIBG uptake, there is evidence that labetalol, which has combined α- and β-adrenergic receptor-blocking properties, reduces ^{123}I-MIBG uptake and may cause release of ^{123}I-MIBG from neurosecretory vesicles (14). Reserpine depletes endogenous catecholamines from granular stores by blocking uptake and has been shown to reduce uptake and increase washout of ^{123}I-MIBG (10,14). Phenylpropanolamine and related sympathomimetics displace intracellular stores of catecholamines and may compete for uptake with ^{123}I-MIBG (10). Hypoglycemia results in a central nervous system–mediated discharge of catecholamines and has been shown to produce rapid washout of ^{123}I-MIBG (10). Because exocytosis is dependent on calcium flux, calcium channel blockers may reduce the neuronal release of ^{123}I-MIBG (10). Reduced ^{123}I-MIBG uptake may be seen with high circulating catecholamine levels as a result of competition for the type-1 uptake mechanism (10). Platelets have an active amine uptake mechanism, and platelet uptake has been reported (10); however, in the literature reviewed, no problems associated with cardiac imaging have been documented.

^{123}I-MIBG CARDIAC IMAGING

Standard Protocol

The standard protocol for ^{123}I-MIBG cardiac imaging requires that drugs that interfere with ^{123}I-MIBG uptake be withheld. A comprehensive listing of prescription and over-the-counter drugs that interfere with ^{123}I-MIBG biodistribution, and the time for which they should be withheld, has been published (14). Thyroid uptake of unbound ^{123}I is blocked with 500 mg of potassium perchlorate given orally 30 min before ^{123}I-MIBG injection (6).

Between 148 MBq (7) and 370 MBq (6) of ^{123}I-MIBG are injected intravenously at rest. Both planar and SPECT im-

ages are acquired 15 min after injection (early) and 4 h after injection (delayed). A 20% window is usually centered over the 159-keV photopeak of ^{123}I for imaging (6). Anterior and 45° left anterior oblique planar images of the chest are acquired using a 128 × 128 matrix (6). SPECT images are acquired using a 64 × 64 matrix over 180°, from the right anterior oblique position to the left posterior oblique position (6). Planar imaging allows for global assessment of cardiac innervation, whereas SPECT allows for regional evaluation (15). A recent study suggested that the use of a medium-energy collimator, rather than a low-energy collimator, for planar imaging may improve the quantitative accuracy and reliability of ^{123}I-MIBG cardiac imaging (16) by reducing scatter from the 0.529-MeV photons that make up 1.4% of the disintegrations of ^{123}I (13,16).

Other Techniques

Dual-isotope imaging techniques, using ^{123}I-MIBG and ^{99m}Tc-sestamibi or ^{201}Tl, that allow ^{123}I-MIBG quantitative analysis to be corrected for the presence of perfusion defects have been described (17–20). These techniques add to the diagnostic value of the study by excluding or compensating for coronary artery disease as a mechanism of reduced uptake (15).

Normal Cardiac Uptake Pattern

Healthy patients normally show good uptake of the radiotracer (Fig. 2). However, uptake may be heterogeneous and be slightly lower in the inferior wall (17,18), most likely because of attenuation (6,7). Activity may also be lower at the apex of the left ventricle than at the base because of the partial-volume effect (7) and, possibly, an imbalance of sympathetic and parasympathetic fibers (15). Homogeneous uniform uptake has also been reported (3). Cardiac function decreases with age, and decreased cardiac responsiveness to β-adrenergic stimulation is one of the most prominently seen changes (6). Decreased cardiac uptake has been shown in elderly patients, and age should likely be considered when assessing ^{123}I-MIBG uptake (21).

Image Markers

Cardiac ^{123}I-MIBG uptake is commonly assessed by viewing the planar images (6). Initial uptake is considered to be a measure of the number and function of norepinephrine transporters in the heart (22). In addition, semiquantitative measures of uptake and washout have been used. The heart-to-mediastinum ratio is obtained from both the early and the delayed planar images (7) and is calculated by taking regions of interest over the myocardium in the anterior view and over the mediastinum in the left anterior oblique view and dividing the average counts per pixel in the myocardium by the average counts per pixel in the mediastinum (6). A high ratio is expected in normal hearts, indicating predominant localization of ^{123}I-MIBG in the myocardium (7). Symptomatic autonomic neuropathy has been associated with profound loss of myocardial uptake (13,22). A recent review has reported an average heart-to-mediastinum ratio of 2.5 ± 0.3 for 96 healthy subjects (7). The washout rate is calculated from the decrease in myocardial counts between the early and the delayed images, normalized to the mediastinal counts (7). The washout rate is believed to reflect adrenergic activity, with increased adrenergic activity being reflected as increased washout (7). Average washout rates of 20% ± 10% have been found in healthy subjects (7). Although increased washout has been observed in diabetic patients (23,24), the clinical importance of this index in diabetes is not clear (25). Planar imaging has limitations, including the superimposition of surrounding structures on cardiac images, which may result in suboptimal images, and possible motion artifacts (6). SPECT overcomes some of these disadvantages (6).

The SPECT data are reconstructed along the short axis, horizontal long axis, and vertical long axis (6). SPECT images are analyzed visually, with regional uptake being scored on a point scale (6). Polar maps are generated from short-axis SPECT images and can be compared with known files of healthy patients (Fig. 3) (6). Studies have shown that sympathetic autonomic neuropathy normally presents in the inferior wall in the early stages of diabetic CAN and then progresses to adjacent segments (3,4,23,26,27). Regional myocardial evaluation using an inferior-to-anterior ratio may therefore be a sensitive index when assessing early diabetic CAN (23). To improve image noise and contrast with SPECT, the dose and imaging time should be maximized (6). SPECT images may be suboptimal if uptake is
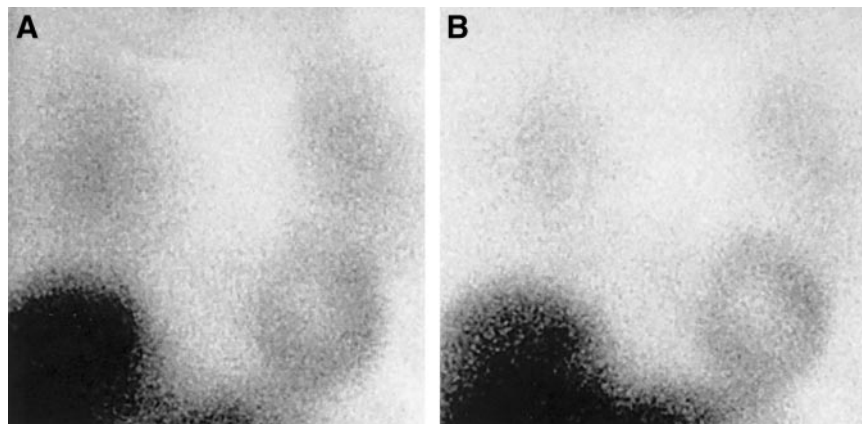

FIGURE 2. Normal ^{123}I-MIBG uptake on early (15 min) planar images (A) and delayed (4 h) planar images (B). (Courtesy of Ignasi Carrió, MD, Barcelona, Spain.)
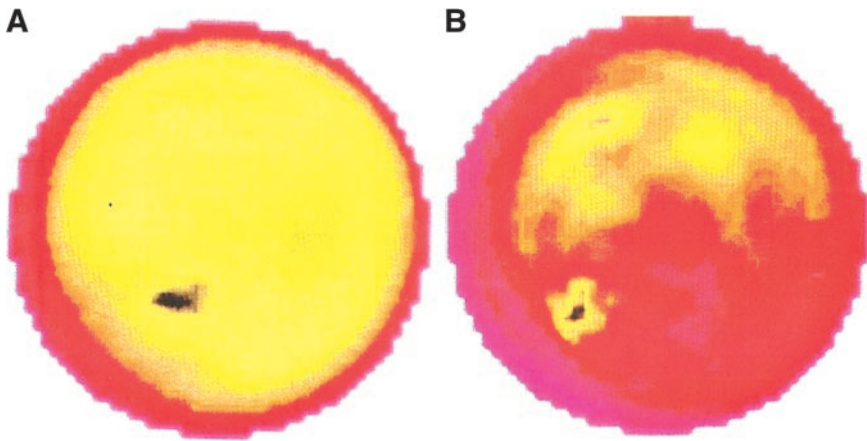
FIGURE 3. Polar map showing normal sympathetic innervation (A) and diabetic sympathetic dysfunction (decreased posterior/inferior uptake) (B). (Courtesy of Oliver Schnell, MD, Munich, Germany.)

severely reduced (6). Differing terminology has been used in the literature to describe the same myocardial region.

Quantitative analysis by these methods is not absolute. With the advent of recent SPECT methods to correct attenuation, more accurate quantification may be possible (6,7,28). Scatter correction may also be of value (6). Despite the factors complicating ^{123}I-MIBG imaging, this technique is believed to reliably detect sympathetic nerve damage in CAN (22).

OTHER METHODS OF EVALUATING CAN

Electrocardiograph-based autonomic function tests have traditionally been used to assess CAN (5,11). These indirect bedside methods include the Valsalva maneuver, heart rate variability, heart rate response to standing, postural fall in blood pressure, and the sustained handgrip test (1,7). Assessed results rely on heart rate or blood pressure responses (20), and autonomic dysfunction is usually defined as an abnormal response to at least 2 of these maneuvers (5,17,29). These methods are noninvasive; however, research has shown that they likely underestimate CAN (5,7,27,29) and reflect changes in parasympathetic function with more sensitivity than they do changes in sympathetic function (7,30). Compared with ^{123}I-MIBG imaging, autonomic function tests are believed to be less sensitive (5,7,11,27,29,30), are nonquantitative, and are unable to evaluate specific anatomic regions (3,11).

The noradrenaline spillover technique can infer noradrenaline release (sympathetic activity) in the heart by using a radioactive noradrenaline infusion and sampling the coronary sinus (6,31). This technique is invasive, requires cardiac catheterization, and is unable to differentiate between increased neuronal release and reduced neuronal uptake (6,31).

PET-based neurotransmission imaging techniques offer a noninvasive, direct, objective, and quantitative way to assess myocardial autonomic function using tracer kinetic modeling (6). Although these techniques are more technically demanding than ^{123}I-MIBG imaging, they can provide more accurate quantitative information on neurotransmitter synthesis and transport, and on receptor density (6,7). However, the clinical utility of these indexes has yet to be determined. The need for an on-site cyclotron is a major factor limiting the routine clinical use of PET to assess autonomic function (31). ^{11}C-Hydroxyephedrine, also an analog of noradrenaline, is commonly used in studies in the United States (32–34). PET research studies have confirmed ^{123}I-MIBG findings in diabetic patients (15).

RESEARCH FINDINGS

The mechanism by which diabetic CAN causes silent myocardial ischemia, infarction, and sudden cardiac death is not fully understood (32) and has been linked to a variety of factors (14). Studies do, however, indicate that even early, preclinical, stages of CAN may be associated with cardiac sympathetic dysfunction that can impair the regulation of coronary blood flow in response to stress (32). This vascular dysfunction is thought to play a role in progressive left ventricular dysfunction in diabetic patients, even in the absence of angiographically assessed coronary artery disease (32). CAN has also been linked to an abnormal left ventricular response to exercise in the absence of ischemic heart disease; such a response may contribute to left ventricular dysfunction before the appearance of irreversible damage and overt heart failure (11,35). Diabetic patients with silent myocardial ischemia have been found to have ^{123}I-MIBG uptake abnormalities suggesting a link between abnormal perception of pain and diabetic CAN (14,18). Stevens et al. (33) have suggested that proximal hyperinnervation together with distal denervation may be associated with arrhythmias and sudden cardiac death in diabetic patients.

Long-term poor glycemic control has been identified as a major factor in the progression of diabetic CAN (29). Small PET defects in myocardial innervation progress with poor glycemic control and regress with good glycemic control, suggesting that good glycemic control in patients with early cardiac denervation may prevent the development of cardiac sympathetic dysinnervation (4). Partial reversal of cardiac

sympathetic denervation as scintigraphically assessed with ^{123}I-MIBG has been shown in patients with newly diagnosed insulin-dependent diabetes mellitus after intensive insulin therapy (2), whereas patients with long-term insulin-dependent diabetes mellitus generally show neither progression nor regression of ^{123}I-MIBG defects (3). Similar results are found in patients with non–insulin-dependent diabetes mellitus (36) after insulin therapy. These findings support the belief that diabetic CAN has a reversible metabolic component and an irreversible structural component, with the irreversible component increasing over time (2), and emphasize the importance of the early diagnosis of diabetic CAN.

Cardiac defects visualized with ^{123}I-MIBG imaging of diabetic patients have been found to be approximately 40% more common than the detection of diabetic CAN by traditional means (7). ^{123}I-MIBG defects may be associated with autonomic neuropathy, arrhythmogenicity due to abnormal regional flow (flow–demand mismatch), left ventricular dysfunction, and microvascular disease, even in the absence of macrovascular disease (7).

THE CLINICAL ROLE OF ^{123}I-MIBG IMAGING IN DIABETIC CAN

Clinically evident autonomic neuropathy in diabetic patients is associated with a poor prognosis (1,37). The early preclinical detection of CAN that is believed to be possible with ^{123}I-MIBG imaging (6,15,26) may thus have an advantage over the less sensitive indirect autonomic reflex testing that is traditionally used (3,5,27). The increased sensitivity of ^{123}I-MIBG imaging over autonomic function testing may also make this method superior for evaluating the effect of metabolic intervention on CAN (29). These factors could permit more focused management of diabetic CAN (17). However, although widely used to characterize diabetic CAN and improve understanding of the natural progression of this disease process, ^{123}I-MIBG imaging has seen routine clinical use only in Japan (7,28).

Although ^{123}I-MIBG is commercially available in Europe, the cost of this procedure appears to have prohibited its clinical use, and ^{123}I-MIBG imaging is used primarily for research in this region. The lack of commercial availability of ^{123}I-MIBG and the high cost are important factors limiting use of this technique in the United States (7,28). ^{123}I-MIBG is currently labeled on site, which requires an investigative new drug application, or is imported from Canada at greater expense (7).

From the literature reviewed, most ^{123}I-MIBG research studies have been performed in Europe and Japan. Most studies have been of relatively few patients and limited to single centers. The largest study assessed 65 diabetic patients (17), but on average the reviewed studies assessed 28 patients. No cost analysis data were available in the literature reviewed.

CONCLUSION

^{123}I-MIBG imaging appears to promise novel, noninvasive clinical information in the early detection of diabetic CAN. Indeed, this technique is used clinically in Japan. However, further clinical studies are needed if ^{123}I-MIBG imaging is to see widespread clinical use in the United States. The clinical usefulness of this technique needs to be validated by large multicenter trials, and the proper positioning of ^{123}I-MIBG imaging in diagnostic algorithms needs to be established (28). The development of ^{99m}Tc-labeled analogues of MIBG (noradrenaline), if feasible, could alleviate the cost and availability issues and lead to greater worldwide clinical use of this technique (6,28). Cost-analysis studies are needed to demonstrate the possible cost-effectiveness of this procedure when total patient outcome is considered (28). ^{123}I-MIBG cardiac imaging continues to be a valuable research tool in diabetes. It remains to be seen whether the cost and availability of this technique will limit the work that still needs to be done to promote it to full clinical status in most regions of the world.

REFERENCES

1. Ewing DJ, Campbell IW, Clarke BF. Assessment of cardiovascular effects in diabetic autonomic neuropathy and prognostic implications. *Ann Intern Med.* 1980;92:308–311.
2. Schnell O, Muhr D, Dresel S, Weiss M, Haslbeck M, Standl E. Partial restoration of scintigraphically assessed cardiac sympathetic denervation in newly diagnosed patients with insulin-dependent (type 1) diabetes mellitus at one-year follow-up. *Diabet Med.* 1997;14:57–62.
3. Schnell O, Muhr D, Weiss M, et al. Three-year follow-up on scintigraphically assessed cardiac sympathetic denervation in patients with long-term insulin-dependent (type 1) diabetes mellitus. *J Diabetes Complications.* 1997;11:307–313.
4. Stevens MJ, Raffel DM, Allman KC, Schwaiger M, Wieland DM. Regression and progression of cardiac sympathetic dysinnervation complicating diabetes: an assessment by C-11 hydroxyephedrine and positron emission tomography. *Metabolism.* 1999;48:92–101.
5. Freeman MR, Newman D, Dorian P, Barr A, Langer A. Relation of direct assessment of cardiac autonomic function with metaiodobenzylguanidine imaging to heart rate variability in diabetes mellitus. *Am J Cardiol.* 1997; 80:247–250.
6. Carrio I. Cardiac neurotransmission imaging. *J Nucl Med.* 2001;42:1062–1076.
7. Patel AD, Iskandrian AE. MIBG imaging. *J Nucl Cardiol.* 2002;9:75–94.
8. Dae MW, O'Connell JW, Botvinick EH, et al. Scintigraphic assessment of regional cardiac adrenergic innervation. *Circulation.* 1989;79:634–644.
9. Camici PG. Imaging of cardiac adrenergic innervation. *Heart.* 2002;88: 209–210.
10. Shapiro B, Gross MD. Radiochemistry, biochemistry, and kinetics of ^{131}I-metaiodobenzylguanidine (MIBG) and ^{123}I-MIBG: clinical implications of the use of ^{123}I-MIBG. *Med Pediatr Oncol.* 1987;15:170–177.
11. Scognamiglio R, Avogaro A, Casara D, et al. Myocardial dysfunction and adrenergic cardiac innervation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol.* 1998;31:404–412.
12. Dae MW, De Marco T, Botvinick EH, et al. Scintigraphic assessment of MIBG uptake in globally denervated human and canine hearts: implications for clinical studies. *J Nucl Med.* 1992;33:1444–1450.
13. Sisson JC, Shapiro B, Meyers L, et al. Metaiodobenzylguanidine to map scintigraphically the adrenergic nervous system in man. *J Nucl Med.* 1987;28:1625–1636.
14. Solanki KK, Bomanji J, Moyes J, Mather SJ, Trainer PJ, Britton KE. A pharmacological guide to medicines which interfere with the biodistribution of radiolabelled meta-iodobenzylguanidine (MIBG). *Nucl Med Commun.* 1992;13:513–521.
15. Giordano A, Calcagni ML, Verrillo A, et al. Assessment of sympathetic innervation of the heart in diabetes mellitus using ^{123}I-MIBG. *Diabetes Nutr Metab.* 2000;13:350–355.

16. Inoue Y, Suzuki A, Shirouza I, et al. Effect of collimator choice on quantitative assessment of cardiac iodine 123 MIBG uptake. *J Nucl Cardiol.* 2003;10:623–632.

17. Langer A, Freeman MR, Josse RG, Armstrong PW. Metaiodobenzylguanidine imaging in diabetes mellitus: assessment of cardiac sympathetic denervation and its relation to autonomic dysfunction and silent myocardial ischemia. *J Am Coll Cardiol.* 1995;25:610–618.

18. Matsuo S, Takahashi M, Nakamura Y, Kinoshita M. Evaluation of cardiac sympathetic innervation with iodine-123-metaiodobenzylguanidine imaging in silent myocardial ischemia. *J Nucl Med.* 1996;37:712–717.

19. Murashima S, Takeda K, Matsumura K, et al. Increased lung uptake of iodine-123-MIBG in diabetics with sympathetic nervous dysfunction. *J Nucl Med.* 1998;39:334–338.

20. Wei K, Dorian P, Newman D, Langer A. Association between QT dispersion and autonomic dysfunction in patients with diabetes mellitus. *J Am Coll Cardiol.* 1995;26:859–863.

21. Estorch M, Carrio I, Berna L, Lopez-Pousa J, Torres G. Myocardial iodine-labeled metaiodobenzylguanidine123 uptake relates to age. *J Nucl Cardiol.* 1995;2:126–132.

22. Glowniak JV. Cardiac studies with metaiodobenzylguanidine: a critique of methods and interpretation of results. *J Nucl Med.* 1995;36:2133–2137.

23. Hattori N, Tamaki N, Hayashi T, et al. Regional abnormality of iodine-123-MIBG in diabetic hearts. *J Nucl Med.* 1996;37:1985–1990.

24. Nagamachi S, Jinnouchi S, Nakahara H, et al. ^{123}I-MIBG myocardial scintigraphy in diabetic patients: relationship to autonomic neuropathy. *Nucl Med Commun.* 1996;17:621–632.

25. Zhao C, Shuke N, Yamamoto W, et al. Comparison of cardiac sympathetic nervous function with left ventricular function and perfusion in cardiomyopathies by ^{123}I-MIBG SPECT and ^{99m}Tc-tetrofosmin electrocardiographically gated SPECT. *J Nucl Med.* 2001;42:1017–1024.

26. Schnell O, Muhr D, Weiss M, Dresel S, Haslbeck M, Standl E. Reduced myocardial ^{123}I-metaiodobenzylguanidine uptake in newly diagnosed IDDM patients. *Diabetes.* 1996;45:801–805.

27. Schnell O, Hammer K, Muhr-Becker D, et al. Cardiac sympathetic dysinnervation in type 2 diabetes mellitus with and without ECG-based cardiac autonomic neuropathy. *J Diabetes Complications.* 2002;16:220–227.

28. DePuey EG, Port S, Wackers FJ, et al. Nonperfusion applications in nuclear cardiology: report of a task force of the American Society of Nuclear Cardiology. *J Nucl Cardiol.* 1998;5:218–231.

29. Ziegler D, Weise F, Langen KJ, et al. Effect of glycaemic control on myocardial sympathetic innervation assessed by [123I]metaiodobenzylguanidine scintigraphy: a 4-year prospective study in IDDM patients. *Diabetologia.* 1998;41:443–451.

30. Kim SJ, Lee JD, Ryu YH, et al. Evaluation of cardiac sympathetic neuronal integrity in diabetic patients using iodine-123 metaiodobenzylguanidine. *Eur J Nucl Med.* 1996;23:401–406.

31. Grassi G, Esler M. How to assess sympathetic activity in humans. *J Hypertens.* 1999;17:719–734.

32. Di Carli MF, Bianco-Batlles D, Landa ME, et al. Effects of autonomic neuropathy on coronary blood flow in patients with diabetes mellitus. *Circulation.* 1999;100:813–819.

33. Stevens MJ, Raffel DM, Allman KC, et al. Cardiac sympathetic dysinnervation in diabetes: implications for enhanced cardiovascular risk. *Circulation.* 1998;98:961–968.

34. Stevens MJ. New imaging techniques for cardiovascular autonomic neuropathy: a window on the heart. *Diabetes Technol Ther.* 2001;3:9–22.

35. Zola B, Kahn JK, Juni JE, Vinik AI. Abnormal cardiac function in diabetic patients with autonomic neuropathy in the absence of ischemic disease. *J Clin Endocrinol Metab.* 1986;63:208–214.

36. Nagamachi S, Jinnouchi S, Kurose T, et al. Serial change in ^{123}I-MIBG myocardial scintigraphy in non-insulin-dependent diabetes mellitus. *Ann Nucl Med.* 2002;16:33–38.

37. Ewing DJ, Campbell IW, Clarke BF. The natural history of diabetic autonomic neuropathy. *Q J Med.* 1980;49:95–108.