Original communication

Diffuse nesidioblastosis as a cause of hyperinsulinemic hypoglycemia in adults: A diagnostic and therapeutic challenge

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Hyperinsulinemic hypoglycemia is caused by uncontrolled insulin release either from neoplastic pancreatic β-cells or from functionally defective β-cells. The latter disorder, which is usually seen in newborns, has been called nesidioblastosis and is divided histopathologically into a focal and diffuse type. In adults, nesidioblastosis is rare, and therefore its histopathologic and clinical features are not well known. In our institution, 4 of 128 adult patients (>3%) suffering from hyperinsulinemic hypoglycemia were found to have diffuse nesidioblastosis. The remaining patients had an insulinoma resected successfully in all but one patient. The diagnosis of diffuse nesidioblastosis was established histopathologically after removing a segment of the distal pancreas. Resection of up to 90% of the pancreas relieved 2 of the 4 patients of their symptoms. We conclude that diffuse nesidioblastosis is rare in adults but may account for more than 3% of patients with hyperinsulinemic hypoglycemia. The histopathologic diagnosis relies predominantly on demonstration of β-cell hypertrophy. The cause of the disease is not known but may be related to defects in the glucose recognition system of the β-cell. Treatment consists of operative reduction of the β-cell mass, but the extent of pancreatic resection required is hard to judge, and there is a thin line between successful treatment, persistence of the disease, and pancreatic endocrine insufficiency. (Surgery 2007;141:179-84.)

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HYPERINSULINEMIC HYPOGLYCEMIA caused by functionally defective β-cells is more common in newborns than in adults. Our knowledge of the pathogenesis of this disease has grown in recent years. A number of genetic abnormalities underlie the disease. The most important are mutations in 2 adjacent genes on chromosome 11, SUR1 and Kir6.2, which encode proteins comprising the ATP-sensitive potassium channel in the cell membrane of the β-cell. Other mutations involve the glucokinase and glutamate dehydrogenase genes. In addition, there may be a random loss of maternally imprinted, growth inhibitory genes at chromosome 11p15 combined with a paternally inherited mutation of the SUR1 or Kir6.2 gene.[1,2] Histologically, the disease is characterized by either diffuse β-cell hypertrophy and β-cell hyperfunction or focal β-cell hypertrophy, hyperplasia, and hyperfunction.[1,3] The pancreatic changes in hyperinsulinemic hypoglycemia have also been referred to as nesidioblastosis, a term coined by Laidlaw[4] in 1938 to describe the neoformation of islets from pancreatic duct epithelium. In 1971, Yakovac et al[5] used this term to report their pancreatic findings in 12 infants with intractable hypoglycemia. Although not fully cor-

Table I. Preoperative clinical diagnostic and imaging characteristics of patients with hyperinsulinemic hypoglycemia due to diffuse nesidioblastosis

Patient no.	1	2	3	4
Gender	m	m	f	m
Age	58	18	31	40
Symptoms	CVA, low bg	Confusion, somnolence	Aphasia, weight gain	Confusion, somnolence
Duration	10 wk	8 y	3 wk	5 m
Diagnostic tests				
Fasting test (bg)	+ (40mg/%)	+ (32 mg/%)	+ (35 mg/%)	+ (30 mg/%)
Diazoxide test	Ø	Ø	Ø	Ø
Ogtt	Ø	Ø	Ø	Ø
Somatostatin test	Ø	Ø	Ø	Ø
Sulfonylurea screen	Ø	Ø	+	Ø
Imaging				
CT abdomen	−	−	−	−
US abdomen	−	−	−	−
Follow-up	8.9 y	11.5 y	7.4 y	9 m

M, male; *f*, female; *CVA*, cerebral vascular accident; *bg*, blood glucose; *wk*, week; *y*, year; *m*, month; +, positive; *oGGT*, oral glucose tolerance test; −, negative; Ø not examined; *CT*, computed tomography; *US*, ultrasound.

rect, nesidioblastosis is used collectively today to designate the pancreatic changes in all persistent hyperinsulinemic conditions not caused by an insulinoma.

In 1975, the first adult with nesidioblastosis was reported.[6] Since then, only a few additional patients have been described, most as individual case reports.[7-11] The histopathologic criteria used to define adult diffuse nesidioblastosis, however, are unclear or even questionable. At our hospital, 4 of 128 patients with hyperinsulinemic hypoglycemia secondary to diffuse nesidioblastosis have been diagnosed, suggesting that adult nesidioblastosis may be more common than assumed. Our report is intended to increase the awareness of the clinical and morphologic features of adult nesidioblastosis and to show how this knowledge can be put to use in the treatment of patients with hyperinsulinemic hypoglycemia.

PATIENTS AND METHODS

Between 1975 and 2005, 128 patients with hyperinsulinemic hypoglycemia were operated on in our hospital. Median age was 47 years, ranging from 14 to 89 years. The majority of patients were female (62%). Most patients presented with typical symptoms of hyperinsulinemic hypoglycemia, ie, hyperadrenergic reactions and neuroglucopenic symptoms such as confusion, amnesia, or blurred vision. Often, a long time had elapsed between the first occurrence of symptoms and the diagnosis of hyperinsulinemic hypoglycemia, even as long as 8 years (Table I). In 2 patients, no cause of the

hyperinsulinemic hypoglycemia could be identified pre- and intraoperatively. In these 2 patients, no samples of the pancreas were taken intraoperatively. One of these patients was operated again 2 years later because of persistent hypoglycemic symptoms. A small insulinoma was identified in the dorsal head of the pancreas. The other patient was lost to follow-up.

The diagnosis of hyperinsulinemic hypoglycemia was based on hypoglycemia (blood glucose concentrations <40 mg/%) in the presence of insulin levels above 6 μl/ml.[12] Prolonged fasting was the most important test. Positive criteria were spike patterns and time until hypoglycemia causing neuroglycopenic syndromes appeared. C peptide levels were determined to exclude exogenous insulin intake. Although done in only 1 of our 4 patients done, patients should be screened for first- and second-generation sulfonylureas by drug screening. With these examinations, factitious hypoglycemia was diagnosed in 2 female patients over 30 years of age. Table I summarizes the characteristics of the patients and preoperative diagnoses (suppression and stimulation tests) in the 4 patients with hyperinsulinemic hypoglycemia due to diffuse nesidioblastosis. One of the four patients (patient 2) described symptoms with fasting (BMI: 20). Compared with the other 124 patients with hyperinsulinemic hypoglycemia, there were no clear differences in clinical symptoms, duration of symptoms, or functional tests.

To localize the source of excessive insulin in the pancreas, we used transcutaneus ultrasonography (US) and computed tomography (CT) of the ab-

Table II. Major histopathologic criteria for the diagnosis of diffuse nesidioblastosis in adults

Major criteria
• Macroscopic, microscopic, and immunohistochemical exclusion of an insulinoma
• Multiple β-cells with enlarged and hyperchromatic nucleus and abundant clear cytoplasm in the majority of the islets
• Islets with normal spatial distribution and regular hormone expression patterns of the various cell types
• No proliferative activity of the Ki-67 antigen (Mib-1) of endocrine cells

domen. The negative results of these studies in our patients are conspicuous (Table I). A false-positive result was obtained in patient 3 by endoscopic US.

For pathologic examination, the resected pancreatic specimens were sectioned at 5-mm intervals. Samples were fixed in 10% buffered formalin and embedded in paraffin. Block step sections (5 μm) were cut and stained conventionally with hematoxylin and eosin and with periodic acid-Schiff reagent. Serial sections from different regions of the specimen were stained immunohistochemically with the avidin-biotin complex (ABC) method using a monoclonal antibody against insulin (BioGenex, San Ramon, Calif, USA; primary dilution 1:40) and polyclonal antibodies against glucagon (BioGenex; dilution 1:60), somatostatin (DakoCytomation, Glostrup, Denmark; dilution 1:200) and pancreatic polypeptide (DakoCytomation, dilution 1:5.000). To ascertain the staining specificity, appropriate controls were performed. Histologic and immunostained pancreatic sections were analyzed by 3 different pathologists blinded to the patient diagnosis. Histologic criteria for nesidioblastosis in adults were used to evaluate the sections (Table II).[13] Age- and sex-matched control pancreatic specimens were used as references.

RESULTS

In the 4 patients, the pancreas was exposed completely during the operation and investigated by bimanual palpation and intraoperative US. Intraoperative US and bimanual palpation were negative in patients 1 to 3. A false-positive image (intraoperative US/bimanual palpation) was obtained in patient 4. In patient 1, a spleen-conserving distal pancreatectomy (beginning to the left of the inferior mesenteric vein) was performed, because intraoperative frozen section analysis led us to suspect nesidioblastosis. In patient 2, despite a positive endoscopic US, intraoperative US and palpation were negative; therefore, a distal pancreatectomy (as above) was performed for histologic sampling. A few days later, after nesidioblastosis had been confirmed histologically, a second operation (subtotal pancreas resection >90%) was performed. In patient

3, a left-sided pancreatic resection was performed (beginning just to the left of the superior mesenteric vein) because nesidioblastosis was suspected (negative localization). In patient 4, a spleen-conserving distal pancreatectomy beginning to the left of inferior mesenteric vein was performed because of the false-positive intraoperative images. Patients 1 and 4 were cured by the operation. In 2 patients (patients 2 and 3), the disease persisted in spite of the pancreatic resection. They developed recurrent hypoglycemia at least once a month with blood sugar levels <40 mg/%. These 4 patients have now been followed for a median of 7 years (range, 9 months to 11.5 years) after pancreatic resection (Table I).

Gross examination and step sectioning of the pancreatic samples excluded the presence of an endocrine neoplasm. The lobular architecture of the exocrine parenchyma was preserved. The pancreatic duct was patent and of normal size. Some of the islets were enlarged (diameters of more than 300 μm) and irregularly shaped (Fig 1, *A* and *B*). A consistent finding in all specimens was the presence of multiple enlarged islet cells, the arrangement of which was a lobulated islet pattern. Cytologically, a considerable subpopulation of endocrine cells showed enlarged and hyperchromatic nuclei (Fig 1, *C* and *D*). These cytologic changes were seen in more than 60% of the islets. Using immunohistochemistry and subsequent staining with hematoxylin, the cytologically conspicuous endocrine cells were identified as β-cells. In contrast, glucagon, somatostatin, and pancreatic polypeptide cells did not give any cytologic abnormalities (Fig. 1, *E* and *F*). The subcellular distribution of insulin and proinsulin was regular, and apparently equal amounts of both antigens were seen. There was no evidence for an expression of the nuclear proliferation antigen ki-67 within the islets. According to this histopathologic analysis, all 4 patients fulfilled the recently described "major criteria" of diffuse nesidioblastosis in adults (Table II).[14] This diagnosis was confirmed in all 4 patients by general pathologists and 2 endocrine pathologist in a double-blind fashion.
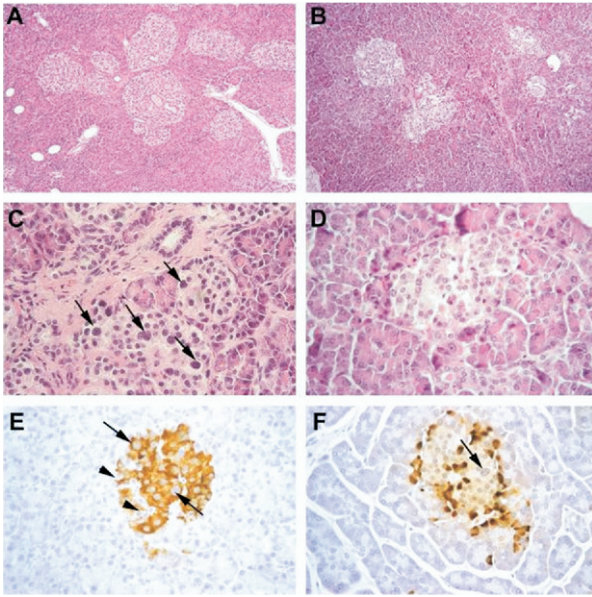
Fig 1. Islet pathology in patients with adult nesidioblastosis in comparison with control pancreatic specimens. Low-power magnification of pancreatic tissue showing increased and irregular-shaped islets in a case of adult nesidioblastosis (**A**) compared to a control (**B**). The high-power magnification reveals small, periductular islet complexes containing cells with hypertrophic nuclei (*arrows* in **C**). **D,** Note regular appearance of islet cells in the control specimen of patients without nesidioblastosis. Immunohistochemical staining for insulin (**E**) and somatostatin (**F**) shows a normal quantitative distribution pattern of both hormones in the islets. The endocrine cells with enlarged nuclei are positive for insulin (*arrows* in **E**) but negative for somatostatin (*arrow* in **F**).

DISCUSSION

In our series of 128 adult patients with hyperinsulinemic hypoglycemia, 123 were found to have an insulinoma, which was resected successfully, and 1 patient without a recognizable insulinoma was lost to follow-up. In 4 patients, nesidioblastosis was confirmed. This diagnosis was established postoperatively based on permanent histologic examination, because there was nothing conspicuous about the prior history or clinical symptoms of our patients with nesidioblastosis compared with patients who had an insulinoma, nor were there any typical peculiarities seen in the functional tests. The results of the preoperative diagnostic procedures (eg, CT, US, and endoscopic US) were negative in all 4 patients. In the literature, only rare cases of a preoperative diagnosis have been reported.[15] A combination of negative, preoperative imaging localization studies, comprehensive operative exploration (completely exposing the pancreas,

bimanual palpation, intraoperative US), and regular follow-up excluded the persistence of a small insulinoma.

The main problem is the lack of imaging techniques sensitive enough to differentiate focal from nonfocal (diffuse) organic hyperinsulinism preoperatively. Neoplasms are often as small as 3 to 10 mm and may escape detection.[12,16-18] Therefore, there is need for diagnostic procedures capable of localizing or excluding an insulinoma definitively. The intraarterial calcium stimulation test (ASVS) is a procedure that allows focal hyperactive β-cell disease to be distinguished from diffuse disease.[11,15,19] ASVS would avoid the necessity of a staged pancreatic resection (eg, blind distal pancreatectomy, endocrine/exocrine insufficiency). In patients with increased insulin secretion from all parts of the pancreas, in the absence of detectable focal insulin production, adult diffuse nesidioblastosis is a likely cause of hyperinsulinemic hypoglycemia. ASVS was not performed, however, routinely for tumor localization in our patients prior to this evaluation.

Of our 4 patients, 2 became normoglycemic after pancreatic resection. In both patients, a distal pancreatectomy sufficed. In 2 patients, there was no amelioration of the hypoglycemia, and they had repeated episodes of hypoglycemia after distal pancreatectomy in one patient and subtotal resection (90%) in the other.

Figure 2 shows our current diagnostic and therapeutic algorithm for patients with hyperinsulinemic hypoglycemia. In case of a positive preoperative localization of a neoplasm (≥1 conclusive image), a local pancreatic resection is performed. The choice of surgical technique depends on the location and site of the neoplasm and its anatomic relation to the pancreatic duct. Enucleation without reconstruction should be undertaken for benign neoplasms and if the pancreatic duct can be preserved. Otherwise pancreatic resection will be performed. If imaging is negative, an ASVS is performed. If regionalization is possible, a local resection is necessary. When localization is impossible, adult nesidioblastosis must be suspected. Initially, a spleen-preserving distal pancreatectomy is recommended. The resected tissue is subjected to standard histologic evaluation, because in frozen sections, enlarged nuclei of the β-cells are difficult to recognize. To establish the diagnosis, histopathologic criteria involve the following: 1. exclusion of an insulinoma by macroscopic, microscopic, and immunhistochemical examinations; 2. multiple β-cells with enlarged and hyperchromatic nuclei and abundant clear cytoplasm; 3. islets with normal spatial distribution of the various cell types; and 4. no
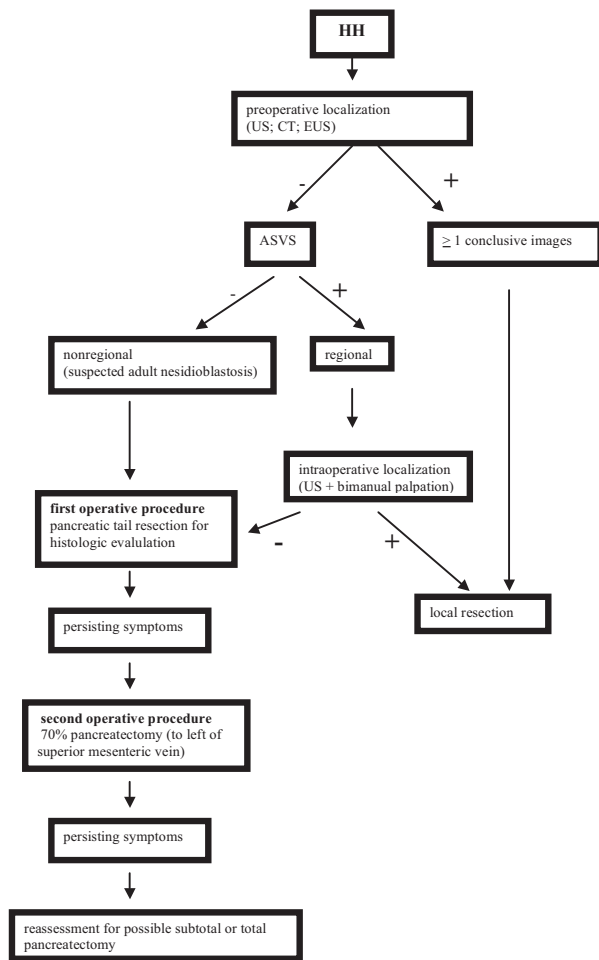
Fig 2. Algorithm for surgical decisions in adult hyperinsulinemic hypoglycemia.

proliferative activity (ki-67 proliferation activity) of endocrine cells (Fig 1).[14] Once the diagnosis of adult nesidioblastosis is established, careful reassessment of the patient is compulsory. When the disease persists, a 70% resection of the pancreatic tissue (extended left-sided resection) is advisable to give the patient a chance to avoid developing diabetes. If the disease continues to persist, a subtotal pancreatectomy (90%) is very likely to control the disease. Although a total pancreatectomy will abolish the hypoglycemic periods, the risk of another pancreatic resection appears to be less than the risks associated with some diabetes after total pancreatectomy.

The limitations of the study must be addressed. In the past, we did not perform drug screening routinely for sulfonylurea in patients with organic hyperinsulinism. All patients were monitored in hospital before the diagnosis, and fasting was carried out under close surveillance. We added drug

screening to our preoperative work-up recently, and one of our patients was tested. This patient was negative for sulfonylurea. Diagnosis of hyperinsulinemic hypoglycemia in our institution is now based on hypoglycemia, increased insulin levels, prolonged fasting, and increased C peptide levels. Also, ASVS was not performed routinely for tumor localization in our patients prior to this evaluation. Nevertheless, we now favor ASVS for regionalization of pathologic insulin release in patients with negative localization imaging.

Our data suggest that about 3% of the patients with hyperinsulinemic hypoglycemia had adult nesidioblastosis. Nesidioblastosis, therefore, seems to account for a substantial fraction of patients with hyperinsulinemic hypoglycemia. The cause of nesidioblastosis in adults is unknown, but may be a genetic defect similar to the molecular changes affecting the insulin secretion signaling process in infants.[20] The treatment is operative resection, but the line between "too much" and "too little" pancreatic resection is narrow and undefined. A prudent approach is warranted and should include informing the patient that multiple procedures may be necessary. There is no optimum procedure for all adult patients with nesidioblastosis, and a second operation involves less risk than the risk of lifetime diabetes mellitus after functional pancreatectomy.

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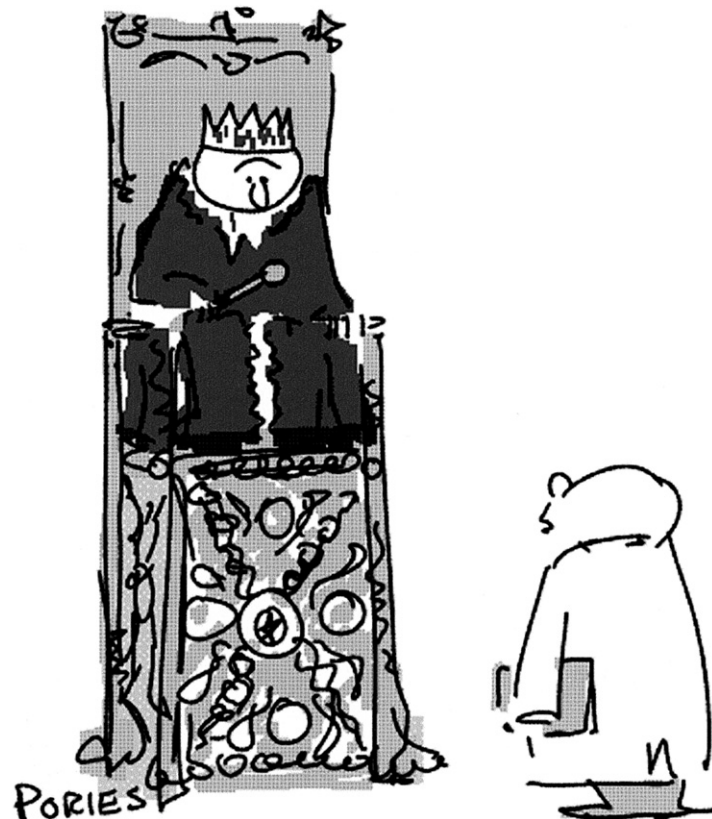
"So how do you like being the chair?"