




# Intraoperative ultrasound imaging in the surgical treatment of congenital hyperinsulinism: prospective, blinded study

A. P. Bjarnesen <sup>1,2</sup>, P. Dahlin<sup>1,2</sup>, E. Globa<sup>3</sup>, H. Petersen <sup>4</sup>, K. Brusgaard<sup>5,6</sup>, L. Rasmussen<sup>2,7</sup>, M. Melikian<sup>8</sup>, S. Detlefsen<sup>2,6,9</sup>, H. T. Christesen <sup>2,6,10</sup> and M. B. Mortensen<sup>1,2,\*</sup>

<sup>1</sup>Upper Gastrointestinal and Hepato-Pancreato-Biliary Section, Department of Surgery, Odense University Hospital, Odense, Denmark

<sup>2</sup>Odense Pancreas Centre, OPAC, Odense University Hospital, Odense, Denmark

<sup>3</sup>Ukrainian Research Centre of Endocrine Surgery, Endocrine Organs and Tissue Transplantation, Paediatric Endocrinology Department, Ministry of Health of Ukraine, Kiev, Ukraine

<sup>4</sup>PET and Cyclotron Unit, Department of Nuclear Medicine, Odense University Hospital, Odense, Denmark

<sup>5</sup>Department of Clinical Genetics, Odense University Hospital, Odense, Denmark

<sup>6</sup>Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

<sup>7</sup>Paediatric Surgery Section, Department of Surgery, Odense University Hospital, Odense, Denmark

<sup>8</sup>Endocrine Research Centre, Department of Paediatric Endocrinology, Moscow, Russia

<sup>9</sup>Department of Pathology, Odense University Hospital, Odense, Denmark

<sup>10</sup>Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark

\*Correspondence to: Department of Surgery, Odense University Hospital, J. B. Winsløvs Vej 4, 5000 Odense C, Denmark (e-mail: michael.bau.mortensen@rsyd.dk)

Presented to the 20th Congress of the European Paediatric Surgeons' Association, Belgrade, Serbia, June 2019, and the Updates in Diagnosis and Management of Hyperinsulinism and Neonatal Hypoglycaemia Conference, Philadelphia, Pennsylvania, USA, September 2019

## Abstract

**Background:** In congenital hyperinsulinism (CHI), preoperative prediction of the histological subtype (focal, diffuse, or atypical) relies on genetics and 6-<sup>18</sup>F]fluoro-L-3,4-dihydroxyphenylalanine (<sup>18</sup>F-DOPA) PET-CT. The scan also guides the localization of a potential focal lesion along with perioperative frozen sections. Intraoperative decision-making is still challenging. This study aimed to describe the characteristics and potential clinical impact of intraoperative ultrasound imaging (IOUS) during CHI surgery.

**Methods:** This was a prospective, observational study undertaken at an expert centre over a 2-year interval. IOUS was performed blinded to preoperative diagnostic test results (genetics and <sup>18</sup>F-DOPA PET-CT), followed by unblinding and continued IOUS during pancreatic resection. Characteristics and clinical impact were assessed using predefined criteria.

**Results:** Eighteen consecutive, surgically treated patients with CHI, with a median age of 5.5 months, were included (focal 12, diffuse 3, atypical 3). Focal lesions presented as predominantly hypoechoic, oval lesions with demarcated or blurred margins. Patients with diffuse and atypical disease had varying echogenicity featuring stranding and non-shadowing hyperechoic foci in three of six, whereas these characteristics were absent from those with focal lesions. The blinded IOUS-based subclassification was correct in 17 of 18 patients; one diffuse lesion was misclassified as focal. IOUS had an impact on the surgical approach in most patients with focal lesions (9 of 12), and in those with diffuse (2 of 3) and atypical (2 of 3) disease when the resection site was close to the bile or pancreatic duct.

**Conclusion:** Uniform IOUS characteristics made all focal lesions identifiable. IOUS had a clinical impact in 13 of 18 patients by being a useful real-time supplementary modality in terms of localizing focal lesions, reducing the need for frozen sections, and preserving healthy tissue and delicate structures.

## Introduction

Congenital hyperinsulinism (CHI) is a heterogeneous disease that causes hypersecretion of insulin by pancreatic  $\beta$ -cells resulting in hypoketotic hypoglycaemia. Despite a low incidence of between 1 in 40 000 and 1 in 50 000 infants, it is the most frequent cause of persistent hypoglycaemia in children. If not diagnosed promptly and treated sufficiently, CHI may cause irreversible neurological damage<sup>1–4</sup>.

CHI is classified into three histological groups: diffuse, focal, and atypical<sup>5,6</sup>. First-line treatment of CHI consists of glucose infusion, nutritional support, diazoxide, and/or octreotide. If this regimen fails to establish normoglycaemia, pancreatic resection can be necessary in early life<sup>1</sup>. In medically non-responsive diffuse or atypical CHI, a subtotal (50–95 per cent) pancreatic resection may lead to normoglycaemia, or facilitate response to medical treatment<sup>1,4,7</sup>. In the event of near-total pancreatectomy, a high risk

Received: May 26, 2020. Accepted: September 4, 2020

© The Author(s) 2020. Published by Oxford University Press on behalf of BJS Society Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

of diabetes is seen at long-term follow-up despite initial normoglycaemia<sup>8</sup>. On the other hand, patients with focal CHI are cured by radical resection of the lesion<sup>1,7</sup>, underscoring the crucial need for a subtype diagnosis as well as correct localization of focal lesions.

Preoperative differentiation between the two major types, focal and diffuse, is governed by genetic testing. Non-dominant, paternal mutations in the adenosine 5'-triphosphate-sensitive potassium ( $K_{ATP}$ ) channel genes *ABCC8* and *KCNJ11* are indicative of focal CHI. Any other genetic findings (including null results) predict diffuse or atypical CHI<sup>9</sup>. If the result is unclear or in favour of focal CHI, preoperative 6-[<sup>18</sup>F]fluoro-L-3,4-dihydroxyphenylalanine (<sup>18</sup>F-DOPA) PET-CT imaging is performed. <sup>18</sup>F-DOPA PET-CT has excellent diagnostic performance, with a sensitivity of 85–100 per cent and specificity of 96–100 per cent in the prediction of focal CHI, but with a lower topographical localization accuracy of 63–100 per cent<sup>10–12</sup>. During surgery, frozen sections are used to confirm the subtype, with a sensitivity and specificity of 94 and 91 per cent respectively in diffuse CHI, and 87 and 100 per cent in focal CHI<sup>13</sup>.

Intraoperative ultrasound imaging (IOUS) has been used routinely during upper gastrointestinal cancer surgery at this centre since 1994. Since 2010, IOUS has been used routinely during CHI surgery, although never assessed in a systematic, prospective and blinded analysis. In a retrospective evaluation of surgically treated patients with focal CHI from 2010 to 2017, IOUS proved valuable for precisely localizing focal lesions during surgery, contributing to a tissue-sparing approach<sup>14</sup>. Further literature on IOUS findings in children with CHI is limited<sup>15,16</sup>. In a blinded, prospective setting, this study sought to describe the IOUS characteristics of CHI tissue, surrounding pancreatic parenchyma and peripancreatic lymph nodes, and to evaluate the potential surgical impact of IOUS.

## Methods

Children diagnosed with CHI, referred to the International Hyperinsulinism Centre, Odense University Hospital (OUH), Denmark, were included. Patients were enrolled between 1 January 2017 and 1 September 2019. Patients who did not have pancreatic surgery were excluded. IOUS during CHI surgery is standard of care at this institution and the present study was considered a qualitative assurance project. Hence, it did not require ethical approval or informed consent according to the Regional Committees on Health Research Ethics for Southern Denmark. The study was approved by the Danish Health Authority (3-3013-2382/1) and the Danish Data Protection Agency (17/40103).

## Setting

The International Hyperinsulinism Centre, OUH, Denmark, is a multidisciplinary, national and international referral centre involving the departments of paediatrics, clinical genetics, nuclear medicine, surgery, paediatric intensive care, and pathology. Operations were carried out at Odense Pancreas Centre, Hepato-Pancreato-Biliary Section, and Section of Paediatric Surgery, Department of Surgery at OUH.

## Surgery and intraoperative ultrasound imaging

Pancreatic surgery was performed by an upper gastrointestinal and hepatopancreatobiliary surgeon (surgeon A) and a paediatric surgeon (surgeon B). Surgeon A, who performed the IOUS, was

blinded to all preoperative investigations and clinical data; surgeon B was not blinded.

All operations were undertaken as open procedures using a supraumbilical transverse incision. After standard exposure, the pancreas was inspected and palpated by surgeon B to detect any potential focal lesion(s). Subsequently, surgeon A performed IOUS by using the stomach as an acoustic window before applying the probe directly to the anterior surface of the pancreas. The gland was scanned from the tail towards the head, using transverse and longitudinal scanning angles. A complete map of IOUS characteristics was recorded using standardized protocols (Tables S1 and S2).

Surgeon A stated what subtype (focal or non-focal) the findings indicated as well as the location of any lesion, after which blinding was abolished. If genetics, <sup>18</sup>F-DOPA PET-CT, and IOUS predicted non-focal CHI, frozen-section analysis was done before resection to verify the subtype. If all three procedures implied focal CHI, frozen sections were obtained only from the resection specimen after enucleation to confirm the diagnosis and, when necessary, completion of resection. Lymph nodes were excised if they were surgically available without increasing patient risk, or if there was doubt whether it was a lymph node or tissue relevant to the CHI pathogenesis (heterotopic CHI tissue).

Unblinded IOUS was used continuously during surgery to avoid damage to relevant structures. In focal CHI, it was also used to identify the margins of the lesion to obtain complete resection while preserving healthy parenchyma. Complete ultrasonographic enucleation of the focal lesion was denoted 'loss of lesion'. Before closure, integrity of the common bile duct (CBD) and main pancreatic duct (MPD) was verified by IOUS. Complications related to the IOUS procedure were recorded at the time. As IOUS was integrated in several steps throughout the surgical procedure, the specific time needed for IOUS was not monitored.

The result of frozen-section analysis was used to determine whether surgery was considered radical, if further resection was needed, or a postoperative observation period was necessary before a final decision could be attained. Final postoperative histology was considered the diagnostic standard. The pathological diagnosis of atypical CHI was defined as non-diffuse and non-focal disease.

Immediately after surgery, surgeon B evaluated the potential impact of IOUS by filling out a questionnaire (Table S3). IOUS was considered to have a positive impact when the lesion was neither macroscopically visible nor palpable but only identifiable on blinded IOUS (criterion 1), or if IOUS gave additional information that changed the surgical approach (such as parenchyma-sparing excision, avoidance of pancreatointestinal anastomosis or less need for frozen sections) compared with preoperative information (criterion 2). IOUS was not used in patients undergoing a second pancreatic operation.

An abdominal drain was placed in all patients to detect, monitor, and treat potential pancreatic fistulas. The amount of fluid and the amylase concentration determined when to remove the drain. Postoperative pancreatic fistulas were classified according to the updated International Study Group of Pancreatic Fistula definition<sup>17</sup>.

## Equipment

PET-CT images were acquired using a GE Discovery™ PET-CT scanner (GE Medical System, Waukesha, Wisconsin, USA) and analysed on a Dexu AW server. IOUS images were generated by a 22-mm high-frequency 7.0–13.0-MHz probe, hockey-stick type

(EUP-054); Hitachi Medical Systems Europe, Steinhausen, Switzerland) connected to a high-end ultrasound platform (EUB-7500A; Hitachi Medical Systems Europe, Steinhausen, Switzerland). <sup>18</sup>F-DOPA PET-CT imaging, genetic analyses, frozen-section analyses, and histological examinations were conducted as described previously<sup>12,14,18</sup>.

## Statistical analysis

IOUS characteristics were analysed using the data collected as indicated in Tables S1 and S2, and the potential impact of IOUS with information recorded as outlined in Table S3. Descriptive statistics were used.

## Results

During the study period, 33 consecutive patients with CHI were referred, of whom 15 were excluded from surgery following successful medical treatment. The remaining 18 patients (9 girls, 9 boys) underwent primary pancreatic surgery and were included in the analysis. Patients were referred from 10 different countries and the median age at time of surgery was 5 months and 19 days (Table 1). Final histology revealed 12 patients with focal, three with diffuse and three with atypical CHI.

## Intraoperative ultrasound imaging characteristics

IOUS was feasible in all patients and performed without any procedure-related complications. The IOUS characteristics of each patient are shown in Table 2. When blinded to all preoperative findings, IOUS correctly predicted focal lesions in all 12 patients and non-focal disease in five of six patients; one patient with diffuse disease was misclassified as having focal CHI (Table 1).

## Focal congenital hyperinsulinism

In patients with histologically confirmed focal CHI, the pancreatic tissue appeared hyperechoic (7), isoechoic (4) or hypoechoic (1) compared with hepatic echogenicity. Compared with the rest of the pancreas, the focal lesions were hypoechoic (9), hyperechoic (1) or mixed type with predominantly hypoechoic appearance (2) (Fig. 1a,b). All lesions were oval (8) or round (4), with blurred (8) or demarcated (4) margins. No lesion was spiculated or distinctly asymmetrical. The median of both the longest and shortest diameters at IOUS was 6 mm, with a range of 3–11 and 2–8 mm respectively. The lesions were situated 0–3 mm from the MPD (12), 0–5 mm from the CBD (for pancreatic head lesions; 4), and in contact with one to three vessels (6). There was no inadvertent damage to the CBD or MPD. Stranding and non-

**Table 1 Patient characteristics and perioperative data for children with congenital hyperinsulinism**

Patient no.	Preoperative					Operative		Postoperative	
	Sex	Country	Age at surgery	Genetics*	<sup>18</sup> F-DOPA PET-CT	IOUS	Frozen section	Genetics*	Histology
1	M	Singapore	1 m, 22 d	Focal	Focal, tail	Focal, tail	Focal	–	Focal, 5 mm
2	M	Kazakhstan	7 m, 1 d	Focal	Focal, head	Focal, head/neck	Focal	–	Focal, 8 mm
3	F	Russia	2 m, 22 d	Focal	Focal, head	Focal, head	Focal	–	Focal, 9 mm
4	F	Hungary	5 m, 11 d	Diffuse/atypical†	Focal, head	Focal, uncinate process	Focal‡	Focal	Focal, 8 mm
5	F	Serbia	7 m, 13 d	Focal	Focal, neck	Focal, neck	Focal	–	Focal, 5 mm
6	M	Sweden	3 m, 2 d	Focal	Focal, tail	Focal, tail	Focal	–	Focal, 9 mm
7	F	Ukraine	5 m, 19 d	Focal	Focal, body	Focal, body	Focal	–	Focal, 11 mm
8	M	Serbia	7 m, 13 d	Focal	Focal, uncinate process	Focal, uncinate process	Focal	–	Focal, 8 mm
9	M	Portugal	4 m, 23 d	Focal	Focal, head	Focal, head	Focal	–	Focal, 7 mm
10	F	Sweden	3 m, 21 d	Focal	Focal, body	Focal, body	Focal	–	Focal, 4 mm
11	M	Ukraine	4 m, 7 d	Focal	Focal, head	Focal, head	Focal	–	Focal, 15 mm
12	M	Portugal	12 m, 9 d	Focal	Focal, body	Focal, body	Focal	–	Focal, 9 mm
13	F	Belarus	5 m, 20 d	Diffuse	Diffuse	Non-focal	Diffuse	–	Diffuse
14	M	Ukraine	9 m, 7 d	Diffuse/focal	Diffuse	Non-focal	Diffuse	–	Diffuse
15	F	Russia	4 m, 17 d	Focal†	Suspicion of focal, tail§	Focal, body and tail¶	Possibly focal#	Diffuse	Diffuse
16	F	Armenia	7 m, 13 d	Focal	Focal lesions in body; diffuse uptake in tail	Non-focal	Normal parenchyma; small area suspicious of focal**	Atypical	Atypical
17	F	Portugal	23 m, 15 d	Diffuse/atypical	Atypical	Non-focal	Atypical	Diffuse/atypical	Atypical
18	M	Ukraine	7 m, 9 d	Diffuse/atypical	Diffuse/atypical	Non-focal	–	–	Atypical

\* Details of predicted subtype by genetics are shown in Table 3.

† Genetic analysis from referring country.

‡ Minor reservations owing to a relatively small number of endocrine cells.

§ Focal tracer uptake, but maximum standardized uptake value ratio below 1.41 (below diagnostic threshold value of 1.44 for focal lesions).

¶ Blinded intraoperative ultrasound imaging (IOUS) regarded the tail lesion as the most distinct and suggested tail resection.

# Small lesion possibly representing focal congenital hyperinsulinism but also a few large, scattered endocrine cell nuclei outside the lesion.

\*\* Frozen sections were not from the resection specimen that proved to contain the major histological findings.

<sup>18</sup>F-DOPA, 6-[<sup>18</sup>F]fluoro-L-3,4-dihydroxyphenylalanine; d, days; m, months.

Table 2 Blinded intraoperative ultrasound characteristics of the pancreas and peripancreatic tissue in congenital hyperinsulinism

Patient no.	IOUS diagnosis	Echogenicity: pancreatic tissue*	Echogenicity: focal lesion†	Characteristics: focal lesion or pancreatic tissue in non-focal CHI	Relationship between focal lesion and surrounding structures
1	Focal	Hyperechoic	Hypoechoic	Tail Oval, 5 × 3 mm Blurred margins	2 mm to MPD Vessels: no contact
2	Focal	Hyperechoic	Hypoechoic	Head and neck Oval, 11 × 8 mm Demarcated margins	1 mm to MPD 0 mm to CBD Vessels: PV, GDA, CHA
3	Focal	Hyperechoic	Hypoechoic	Head Oval, 3 × 2 mm Blurred margins	0 mm to MPD 0.5 mm to CBD Vessels: no contact
4	Focal	Hyperechoic	Hypoechoic	Uncinate process Oval, 10 × 7 mm Blurred margins	0 mm to MPD 8 mm to CBD Vessels: SMV
5	Focal	Isoechoic	Mixed type, predominantly hypoechoic	Neck Oval, 5 × 3 mm Blurred margins	1 mm to MPD 5 mm to CBD Vessels: PV
6	Focal	Isoechoic	Hypoechoic	Tail Oval, 11 × 8 mm Demarcated margins	0 mm to MPD Vessels: no contact
7	Focal	Hypoechoic	Hypoechoic	Body Oval, 11 × 8 mm Demarcated margins	0 mm to MPD Vessels: SMV
8	Focal	Hyperechoic	Hypoechoic	Uncinate process Oval, 6 × 4 mm Demarcated margins	3 mm to MPD 5 mm to CBD Vessels: SMA and SMV
9	Focal	Isoechoic	Hypoechoic	Head Round, 6 × 6 mm Blurred margins	1 mm to MPD 5 mm to CBD Vessels: no contact
10	Focal	Hyperechoic	Hypoechoic	Body Round, 4 × 4 mm Blurred margins	2 mm to MPD Vessels: SV
11	Focal	Isoechoic	Hyperechoic	Head Round, 11 × 8 mm Blurred margins	0.5 mm to MPD 2 mm to CBD Vessels: no contact
12	Focal	Hyperechoic	Mixed type, predominantly hypoechoic	Body Round, 6 × 6 mm Blurred margins	0 mm to MPD Vessels: no contact
13	Non-focal	Hypoechoic	–	Stranding and non-shadowing hyperechoic foci	–
14	Non-focal	Hyperechoic	–	Stranding	–
15	Focal	Isoechoic	Hypoechoic	Tail Round, 5 × 4 mm Demarcated margins One smaller lesion in body‡	2 mm to MPD Vessels: SA (lesion in tail), SMA and SMV (lesions in neck)
16	Non-focal	Hyperechoic head, mixed type in body and tail	–	Head: stranding and non-shadowing hyperechoic foci	–
17	Non-focal	Mixed type	–	–	–
18	Non-focal	Isoechoic	–	Appeared normal	–

\* Of the entire pancreas in non-focal congenital hyperinsulinism (CHI), and of perilesional pancreatic tissue in focal CHI; hepatic tissue as reference standard.

† Pancreatic tissue as reference standard.

‡ Blinded intraoperative ultrasound imaging (IOUS) regarded the tail lesion as the most distinct and suggested tail resection.

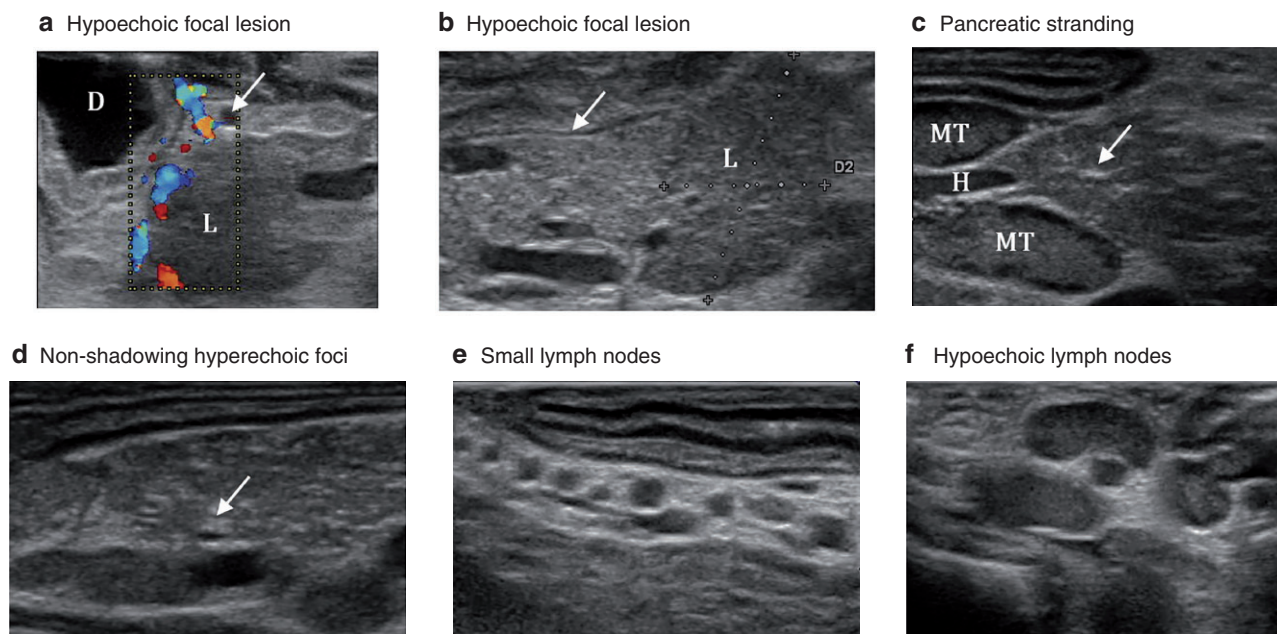
MPD, main pancreatic duct; CBD, common bile duct; PV, portal vein; GDA, gastroduodenal artery; CHA, common hepatic artery; SMV, superior mesenteric vein; SMA, superior mesenteric artery; SV, splenic vein; SA, splenic artery.

shadowing hyperechoic foci were never observed in patients with focal CHI.

'Loss of lesion' was observed in all except one patient, in whom IOUS identified a focal lesion in the uncinate process. However, IOUS and <sup>18</sup>F-DOPA PET-CT were unable to show remnant CHI tissue in the wall of the duodenum that was recognized by palpation and frozen-section analysis during attempted enucleation. Frozen-section analysis indicated focal CHI, but with a minor reservation because of a relatively small number of

endocrine cells (Table 1). The surgeons acknowledged that a complete enucleation would necessitate a Whipple's procedure, and it was decided to stop the operation and await supplementary genetics, final histology and clinical response. Focal CHI was ultimately verified and a Whipple's procedure was subsequently performed as the patient had not responded clinically.

All patients with focal CHI had a paternal  $K_{ATP}$  channel mutation and focal uptake on preoperative <sup>18</sup>F-DOPA PET-CT. In one patient, a second hit of paternal uniparental disomy of



**Fig. 1** Intraoperative ultrasound images in congenital hyperinsulinism

**a** Hypoechoic focal congenital hyperinsulinism (CHI) lesion (L) with blurred margins. The duplex signal shows microblood vessel supply of the lesion; the duodenum (D) and gastroduodenal artery (arrow) are shown (patient 4). **b** Hypoechoic focal CHI lesion (L) with demarcated margins; the main pancreatic duct (arrow) is shown (patient 7). **c** Diffuse CHI with pancreatic stranding (white arrow); two mixed-type lymph nodes (MT) with a hyperechoic core surrounded by a hypoechoic outer rim, and one hypoechoic lymph node (H) are shown (patient 13). **d** Diffuse CHI with non-shadowing hyperechoic foci (white arrow) (patient 13). **e** Small lymph nodes adjacent to vessels between the stomach and pancreas (patient 5). **f** Hypoechoic lymph nodes embedded in the pancreatic parenchyma (patient 5).

**Table 3** Detailed results of genetic testing in surgically treated children with congenital hyperinsulinism

Patient no.	Mutation	Prediction of subtype
1	<i>ABCC8</i> , c.4480C>T, p.(Arg1494Trp), paternal	Focal
2	<i>ABCC8</i> , c.1332G>T, p.(Gln444His), paternal	Focal
3	<i>ABCC8</i> , c.1032C>G, p.(Tyr344*), paternal	Focal
	<i>KCNJ11</i> , c.1096G>A, p.(Gly366Arg), paternal (rare variant, uncertain significance)	Focal
4	Normal CHI genetic panel†	Diffuse/atypical
	Postoperative: <i>ABCC8</i> , c.4415-13G>A, p.?(intronic), paternal	Focal
5	<i>ABCC8</i> , c.4100C>A, p.(Ala1367Asp), paternal	Focal
6	<i>ABCC8</i> , c.4317C>G, p.(Asn1439Lys), novel, paternal	Focal
7	<i>ABCC8</i> , c.2480del, p.(Gly827Alafs*38), paternal	Focal
8	<i>ABCC8</i> , c.3643C>T, p.(Arg1215Trp), paternal	Focal
9	<i>ABCC8</i> , c.2800C>T, p.(Arg934*), heterozygous, non-maternal (paternal DNA n.a.)	Focal‡
10	<i>ABCC8</i> , c.1630 + 1G>T, paternal	Focal
11	<i>KCNJ11</i> , c.629T>C, p.(Ile210Thr), novel, paternal	Focal
12	<i>ABCC8</i> , c.1467 + 1G>A; p.?, paternal	Focal
13	<i>ABCC8</i> , c.695G>A, p.(Trp232*), homozygous	Diffuse
14	<i>ABCC8</i> , c.4518C>G, p.(Asp1506Glu), heterozygous, <i>de novo</i>	Diffuse/focal§
15	<i>ABCC8</i> , c.4017G>A, p.(Trp1339*), non-maternal (paternal DNA n.a.)†	Focal
	Postoperative: additional <i>ABCC8</i> insertion, c.674_675insCACGAAGTAGCA, p.(Leu225_Ser226insThrLys*), maternal	Diffuse
16	<i>ABCC8</i> , c.4489G>A, p.(Val1497Met), paternal	Focal
	Postoperative: segmental, mosaic paternal uniparental disomy of 11p15 in pancreatic tissue (not present in blood)	Atypical
17	Normal CHI genetic panel	Diffuse/atypical
	Postoperative: normal Beckwith–Wiedemann analysis in blood and pancreatic tissue	Diffuse/atypical
18	Normal CHI genetic panel	Diffuse/atypical

Genetic testing was done before operation, if not otherwise indicated. *ABCC8* according to GenBank accession number NM\_001287174.1, *KCNJ11*; NM\_000525.3.

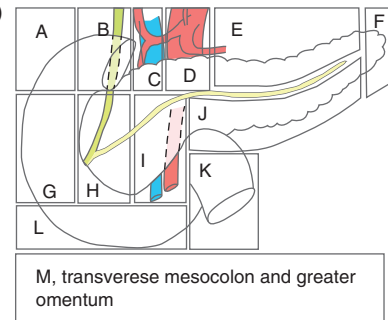
† Genetic analysis from referring country.

‡ A heterozygous, non-maternal mutation without paternal DNA analysis usually predicts focal type if the father is healthy and the child has severe hyperinsulinism. In this patient, paternal uniparental disomy of chromosome 11p15 was found in tissue from the focal lesion, consistent with the two-hit hypothesis of focal lesions.

§ A heterozygous *de novo* adenosine 5'-triphosphate-sensitive potassium channel mutation either predicts diffuse congenital hyperinsulinism (CHI) (dominant effect) or focal CHI (if the mutation is on the paternal allele despite no mutation in the father).

n.a., Not available.

Patient no.	Location													Total	Size (mm)	
	A	B	C	D	E	F	G	H	I	J	K	L	M			
1					2				1	2					5	n.a.
2		2	1		2	2			2	2					11	3–14
3				1					2	6					9	n.a.
4		1							1	2			1		5	n.a.
5			1	1	6	3	1	1		7			1		21	3–15
6			1							3	3			1	8	4–8
7								n.a.							n.a.	n.a.
8			1		1		1			2		1	2		8	3–11
9		1	1		1			1	2	2					8	3–8
10				1	1			1	2						5	3–5
11		1			5	2		1	1				2		12	3–10
12		1	1		4	3	1	1	3	4					18	3–8
13			2	1	3			2	1	3					12	2–6
14			1		5	4			3	4					17	3–10
15				1						4					5	n.a.
16		1	2		2	1		2	1	2			5		16	2–30
17			1		5		1	2	2	30	1	2			44	3–11
18				1	2			1	1						5	2–7
Total	0	7	12	6	39	15	4	19	15	76	2	9	5	209		



**Fig. 2** Location, number and size of lymph nodes detected at intraoperative ultrasound imaging

n.a., Not available.

chromosome 11p15 was found in tissue analysis from the focal lesion (Table 3).

### Diffuse congenital hyperinsulinism

In the three patients with histologically confirmed diffuse CHI, pancreatic tissue was recognized as hypoechoic, hyperechoic or isoechoic compared with hepatic echogenicity. In one patient, non-shadowing hyperechoic foci and stranding were found in the body and tail, and to a lesser extent in the head (Fig. 1c and d); in another, stranding was seen throughout the pancreas. No nodules suspicious of focal lesions were found in either of these patients.

Blinded IOUS misclassified the disease in patient 15 as focal because of two hypoechoic lesions in the body and tail, with absence of other features. The lesion in the tail was more distinct and surgically available, and the suggestion based on the blinded IOUS data was to perform a tail resection, and await clinical response and histological examination before more extensive surgery. Revelation of the <sup>18</sup>F-DOPA PET-CT data supported the IOUS findings as only the lesion in the tail was visible on both PET and CT, but still the maximum standardized uptake value ratio only reached 1.41, below the diagnostic threshold of 1.44 for focal lesions. Genetics from the referring country also suggested focal CHI because of a non-maternal truncating ABCC8 mutation. Consequently, the surgical approach based on the blinded IOUS findings was maintained. Blood glucose normalization was only transient and a second operation was performed. The final histology showed diffuse CHI with an unusual area in the tail of intense ductuloinsular complexes with perilobular fibrosis. A postoperative genetic investigation identified an additional maternal ABCC8 insertion, compatible with compound heterozygosity and diffuse CHI (Table 3).

### Atypical congenital hyperinsulinism

In one patient, the echogenicity of the body and tail region was of mixed type, whereas the head was hyperechoic, with signs of stranding and non-shadowing hyperechoic foci creating a clear ultrasonographic separation line. Macroscopically, the body and

tail also differed from the head because it was enlarged. No nodules were found. The body and tail were resected. Genetic analyses showed segmental, mosaic paternal uniparental disomy of 11p15 in the resected pancreatic tissue, with no other mutations in the CHI next-generation sequencing genetic panel (Table 3). There were no signs of Beckwith-Wiedemann syndrome in the blood, saliva or resected lymph nodes.

A second patient also had mixed-type echogenicity but showed none of the other features, and was genetically unexplained in both blood and pancreatic tissue analyses. The pancreatic tissue of the third patient had normal echogenic features.

### Lymph nodes and other findings

A total of 209 peripancreatic lymph nodes were identified (Fig. 2). Nodes mainly appeared hypoechoic compared with pancreatic tissue (Fig. 1e,f) but 57 had mixed echogenicity, with a hyperechoic core and a hypoechoic outer rim (Fig. 1c). Histological examination of 22 nodes showed non-specific reactive changes in 16 and normal lymphatic tissue in six. All showed no sign of CHI tissue.

Among all 18 patients, the diameter of the MPD ranged from less than 0.5 to 1.0 mm, and the CBD from less than 0.5 to 2.5 mm. None of the patients presented with ascites. No calcifications were identified within the parenchyma or in the ducts.

### Impact of intraoperative ultrasound imaging

In five of 12 patients with focal CHI, blinded IOUS identified lesions that were neither palpable nor macroscopically visible (criterion 1) (Table 4). IOUS was used in all patients with focal CHI to identify the margins of the focus, and in nine of 12 it changed the surgical approach by preventing continuous piecemeal resections and time-consuming frozen-section analyses (criterion 2). In one patient, IOUS was also used to estimate the depth of the focal lesion on the posterior surface of the pancreas close to the portal vein, which would otherwise have required division of the pancreas to explore this region (criterion 2). In 12 patients, IOUS was used to identify and allow resection close to either the CBD or MPD. In these patients, final IOUS after resection was used to

**Table 4 Macroscopic operative findings and impact of intraoperative ultrasound imaging**

Patient no.	Lesion visible	Lesion palpable	Type of surgery	Impact criteria*
1	No	Yes	Resection of tip of tail	None
2	No	No	Enucleation in head/neck	1, 2
3	No	No	Enucleation in head	1, 2
4	Yes	Yes	1: enucleation in uncinata process 2: Whipple's procedure†	2
5	No	No	Enucleation in neck	1, 2
6	Yes	Yes	Enucleation in tail	2
7	Yes	Yes	Enucleation in body	2
8	No	No	Enucleation in uncinata process	1, 2
9	No	Yes	Enucleation in head	None
10	No	No	Enucleation in body	1, 2
11	No	Yes	Enucleation in head	2
12	Yes	Yes	Enucleation in body	None
13	–	–	Body and tail resection	2
14	–	–	Body and tail resection	2
15	–	–	1: resection of tail (assumed focal) 2: body and tail resection (diffuse disease)	None
16	–	–	Body and tail resection	2
17	–	–	Tail resection	2
18	–	–	Body and tail resection	None

\* Criterion 1: the lesion was neither visible nor palpable but only identifiable on blinded intraoperative ultrasound imaging (IOUS); criterion 2: IOUS gave additional information that changed the surgical approach (such as parenchyma-sparing excision, avoidance of pancreaticoenteric anastomosis or less need for frozen sections) compared with preoperative information.

† Owing to heterotopic congenital hyperinsulinism tissue in duodenum wall.

ensure the integrity of the ducts (criterion 2). IOUS gave additional information that changed or minimized the surgical approach in 13 of 18 patients (9 focal, 2 diffuse, 2 atypical).

No frozen sections were used to localize the focal lesions, a maximum of one was used to guide total removal of the lesion, and no more than two frozen sections were needed to confirm the diagnosis in each patient.

Stable normoglycaemia was achieved during the hospital stay in all patients with focal CHI after one (11) or two (1) operations. One patient with atypical CHI achieved stable normoglycaemia after one operation, and one with diffuse disease after two procedures. The latter two patients were discharged without medication, but blood glucose measurements were recommended. The remaining patients with diffuse and atypical CHI continued with nutritional and medical treatment after surgery.

## Discussion

This structured, prospective, and blinded collection of IOUS data from 18 patients CHI identified uniform presentation of focal lesions: predominantly hypoechoic, oval or round, with demarcated or slightly blurred margins. IOUS was able to detect such lesions in all parts of the pancreas down to a diameter of only 2 mm. The patients with diffuse and atypical disease presented with varying patterns of echogenicity; stranding and non-shadowing hyperechoic foci were common characteristics in three of these six patients, but absent from those with focal CHI. One patient with diffuse CHI was misclassified as having focal disease because of hypoechoic areas, whereas the subclassification was correct in the remainder. IOUS added information that improved perioperative evaluation and changed the surgical approach in 13 of 18 patients.

The prerequisite for safe and successful focal CHI surgery is to find and resect the lesion, while preserving healthy tissue without damaging important neighbouring structures. A previous study<sup>15</sup> of IOUS in five patients with focal CHI visualized lesions as typically hypoechoic, with a blurred margin and a size

of 5–17 mm. This was similar to the present findings, supporting the concept that focal lesions have a uniform presentation that makes them identifiable on IOUS. In contrast, a retrospective study<sup>19</sup> focusing on surgical outcome in CHI found that most focal lesions had similar echogenicity to normal pancreatic tissue (hyperechoic), although IOUS was used only in selected patients in whom genetics, but not <sup>18</sup>F-DOPA PET-CT, indicated a focal lesion.

Although <sup>18</sup>F-DOPA PET-CT is the cornerstone of preoperative imaging, from a surgical point of view there are still challenges. <sup>18</sup>F-DOPA PET-CT reflects functional tracer uptake with an indefinite margin of the radiation load. It cannot visualize the precise morphology of the lesion, and relationships with critical structures such as the CBD, MPD or major vessels. It is not easy to localize a focal lesion in the three-dimensional pancreas based on recall of a two-dimensional image, particularly when a focal lesion does not exhibit macroscopically visible or palpable features to distinguish it from normal pancreatic tissue.

IOUS enhanced spatial sensation, providing continuous direct feedback to the surgeon. In the present study, IOUS identified the correct extent of 11 of 12 focal lesions, of which five were neither macroscopically visible nor palpable. The exception was a single patient with heterotopic pancreatic tissue in the wall of the duodenum, which was not recognized by IOUS or <sup>18</sup>F-DOPA PET-CT.

Continuous IOUS guidance reduced the need for some frozen sections. When preoperative investigations lead to suspicion of a focal lesion, three superficial biopsies from the head, body, and tail have been advocated at the outset, to rule out diffuse disease<sup>7</sup>. Multiple frozen sections have also been recommended to find the focal lesion and confirm radical resection<sup>7,13,19</sup>. With use of IOUS, no frozen sections were needed to localize a focal lesion and a maximum of one was needed to guide its total removal. Reliance on IOUS has meant that the median number of frozen sections per patient used at this centre from 2010 to 2017 has fallen to 2<sup>14</sup>, compared with a range of 2–8 frozen sections to localize the focal lesion and 10–29 to complete the resection in a study that did not use IOUS<sup>20</sup>. The present study used IOUS to

confirm complete ultrasonographic removal of CHI tissue (loss of lesion) instead of taking biopsies from the remaining (presumably healthy) pancreas. As each analysis of a frozen section takes at least 20 min, the entire procedure can be lengthy, depending on the number needed.

Stranding was observed in three of six patients with non-focal disease. Non-shadowing hyperechoic foci were seen in two of these six patients. These features correlated with histological findings of chronic pancreatitis<sup>21</sup>, recognized as minor criteria in the Rosemont criteria for the endoscopic ultrasound diagnosis<sup>22</sup>. Microscopy of the pancreatic specimens from these three patients showed perilobular fibrosis, and in one patient there was chronic inflammation with many eosinophilic granulocytes—all changes commensurate with early features of chronic pancreatitis. No nodules, stranding or non-shadowing hyperechoic foci were seen in any of the patients with focal disease away from the lesion.

A single patient was misclassified as having focal disease by IOUS, <sup>18</sup>F-DOPA PET-CT, and frozen-section analyses. Misclassification may be explained by the histological examination that showed foci of perilobular fibrosis surrounding ductuloinsular complexes, resulting in a false perception of a small localized lesion. This patient had unique histological and genetic features. The pancreas was macroscopically enlarged in the body and tail, and the patient was diagnosed with somatic genetic changes (paternal uniparental disomy of chromosome 11p15); if present in the germline, this would lead to Beckwith–Wiedemann syndrome, where organ overgrowth may lead to cancer<sup>23</sup>. Somatic paternal uniparental disomy of chromosome 11p15 was also found in one of the patients with focal CHI, showing that somatic genetic changes with resulting overgrowth may be related to both focal CHI and more widespread, atypical CHI<sup>24</sup>.

In general, patients with non-focal CHI had more lymph nodes detected than those with focal disease. This observation is important because some lymph nodes may mimic hypoechoic focal lesions, and smaller lymph nodes tended to be embedded in the soft pancreatic parenchyma during IOUS.

IOUS is safe, cheap and simple to use during surgery, although limited by the need to acquire technical and interpretive skills. The strengths of the present study include the prospective design, a consecutive patient cohort, blinding of the IOUS operator, and detailed data collection. Limitations include the relatively small data set, and the absence of external validity of IOUS and its impact. Evaluation of impact is difficult, as it may be hard to quantify the sensation of performing safer surgery by having more information about important related structures or a reduced need for frozen sections when guided by IOUS.

IOUS has shown relevant clinical impact in patients with focal CHI lesions and in most of those with diffuse or atypical CHI, when the resection site was close to the CBD or MPD. These real-time images changed the surgical approach, leading to parenchyma-sparing excisions with little risk of pancreatointestinal anastomosis and minimized use of frozen sections. IOUS should be regarded as a supplementary modality to differentiate CHI types, localize focal lesions, and improve surgical decision-making.

## Acknowledgements

H.T.C. and M.B.M. contributed equally to this work. This study was not preregistered at an institutional registry. The authors thank all referring institutions from several countries for their

collaboration in management of the patients: S. Chandran (Department of Neonatology, KK Women's and Children's Hospital, Singapore); Ü. Méder (Department of Paediatrics, Semmelweis University, Budapest, Hungary); T. Milenkovic (Department of Endocrinology, Mother and Child Healthcare Institute of Serbia 'Dr Vukan Čupić', Belgrade, Serbia); K. Kvernebo Sunnergren (Department of Paediatrics, Länssjukhuset Ryhov, Jönköping, Sweden); V. Martins (Department of Paediatrics, Centro Hospitalar de Trás-os-Montes e Alto Douro, Vila Real, Portugal); G. Bayburdyan (Endocrinology Clinic, Yerevan State Medical University, Muratsan University Hospital, Armenia); and C. Limbert (NOVA Medical School and Paediatric Endocrinology and Diabetes, Hospital Dona Estefania, Lisbon, Portugal).

Disclosure. The authors declare no conflict of interest.

## References

- Yorifuji T, Horikawa R, Hasegawa T, Adachi M, Soneda S, Minagawa M *et al*. Clinical practice guidelines for congenital hyperinsulinism. *Clin Pediatr Endocrinol* 2017;**26**:127–152
- Arnoux JB, Verkarre V, Saint-Martin C, Montravers F, Brassier A, Valayannopoulos V *et al*. Congenital hyperinsulinism: current trends in diagnosis and therapy. *Orphanet J Rare Dis* 2011;**6**:63
- Helleskov A, Melikyan M, Globa E, Shcherderkina I, Poertner F, Larsen AM *et al*. Both low blood glucose and insufficient treatment confer risk of neurodevelopmental impairment in congenital hyperinsulinism: a multinational cohort study. *Front Endocrinol* 2017;**8**:156
- Yorifuji T. Congenital hyperinsulinism: current status and future perspectives. *Ann Pediatr Endocrinol Metab* 2014;**19**:57–68
- Demirbilek H, Rahman SA, Buyukyilmaz GG, Hussain K. Diagnosis and treatment of hyperinsulinaemic hypoglycaemia and its implications for paediatric endocrinology. *Int J Pediatr Endocrinol* 2017;**2017**:9
- Rahier J, Guiot Y, Sempoux C. Morphologic analysis of focal and diffuse forms of congenital hyperinsulinism. *Semin Pediatr Surg* 2011;**20**:3–12
- Barthlen W, Mohnike W, Mohnike K. Techniques in pediatric surgery: congenital hyperinsulinism. *Horm Res Paediatr* 2011;**75**:304–310
- Arya VB, Senniappan S, Demirbilek H, Alam S, Flanagan SE, Ellard S *et al*. Pancreatic endocrine and exocrine function in children following near-total pancreatectomy for diffuse congenital hyperinsulinism. *PLoS One* 2014;**9**:e98054
- Galcheva S, Al-Khawaga S, Hussain K. Diagnosis and management of hyperinsulinaemic hypoglycaemia. *Best Pract Res Clin Endocrinol Metab* 2018;**32**:551–573
- Laje P, States LJ, Zhuang H, Becker SA, Palladino AA, Stanley CA *et al*. Accuracy of PET/CT scan in the diagnosis of the focal form of congenital hyperinsulinism. *J Pediatr Surg* 2013;**48**:388–393
- Zani A, Nah SA, Ron O, Totonelli G, Ismail D, Smith VV *et al*. The predictive value of preoperative fluorine-18-L-3,4-dihydroxyphenylalanine positron emission tomography-computed tomography scans in children with congenital hyperinsulinism of infancy. *J Pediatr Surg* 2011;**46**:204–208
- Christiansen CD, Petersen H, Nielsen AL, Detlefsen S, Brusgaard K, Rasmussen L *et al*. 18F-DOPA PET/CT and 68Ga-DOTANOC PET/CT scans as diagnostic tools in focal congenital hyperinsulinism: a blinded evaluation. *Eur J Nucl Med Mol Imaging* 2018;**45**:250–261
- Suchi M, Thornton PS, Adzick NS, MacMullen C, Ganguly A, Stanley CA *et al*. Congenital hyperinsulinism: intraoperative biopsy interpretation can direct the extent of pancreatectomy. *Am J Surg Pathol* 2004;**28**:1326–1335



14. Bendix J, Laursen MG, Mortensen MB, Melikian M, Globa E, Detlefsen S *et al.* Intraoperative ultrasound: a tool to support tissue-sparing curative pancreatic resection in focal congenital hyperinsulinism. *Front Endocrinol* 2018;**9**:11
15. von Rohden L, Mohnike K, Mau H, Eberhard T, Mohnike W, Blankenstein O *et al.* Intraoperative sonography: a technique for localizing focal forms of congenital hyperinsulinism in the pancreas. *Ultraschall Med* 2011;**32**:74–80
16. von Rohden L, Mohnike K, Mau H, Eberhard T, Mohnike W, Blankenstein O *et al.* Visualization of the focus in congenital hyperinsulinism by intraoperative sonography. *Semin Pediatr Surg* 2011;**20**: 28–31
17. Eshmunov D, Schneider MA, Tschuor C, Raptis DA, Kambakamba P, Muller X *et al.* Systematic review and meta-analysis of postoperative pancreatic fistula rates using the updated 2016 International Study Group Pancreatic Fistula definition in patients undergoing pancreatic resection with soft and hard pancreatic texture. *HPB (Oxford)* 2018;**20**:992–1003
18. Rasmussen AG, Melikian M, Globa E, Detlefsen S, Rasmussen L, Petersen H *et al.* The difficult management of persistent, non-focal congenital hyperinsulinism: a retrospective review from a single, tertiary center. *Pediatr Diabetes* 2020;**21**:441–455
19. Adzick NS, De Leon DD, States LJ, Lord K, Bhatti TR, Becker SA *et al.* Surgical treatment of congenital hyperinsulinism: results from 500 pancreatectomies in neonates and children. *J Pediatr Surg* 2019;**54**: 27–32
20. Barthlen W, Varol E, Empting S, Wieland I, Zenker M, Mohnike W *et al.* Surgery in focal congenital hyperinsulinism (CHI)—the ‘Hyperinsulinism Germany International’ experience in 30 children. *Pediatr Endocrinol Rev* 2016;**14**:129–137
21. Varadarajulu S, Eltoun I, Tamhane A, Eloubeidi MA. Histopathologic correlates of noncalcific chronic pancreatitis by EUS: a prospective tissue characterization study. *Gastrointest Endosc* 2007;**66**:501–509
22. Catalano MF, Sahai A, Levy M, Romagnuolo J, Wiersema M, Brugge W *et al.* EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. *Gastrointest Endosc* 2009;**69**:1251–1261
23. Kalish JM, Boodhasingh KE, Bhatti TR, Ganguly A, Conlin LK, Becker SA *et al.* Congenital hyperinsulinism in children with paternal 11p uniparental isodisomy and Beckwith–Wiedemann syndrome. *J Med Genet* 2016;**53**:53–61
24. Flanagan SE, Kapoor RR, Smith VV, Hussain K, Ellard S. Paternal uniparental isodisomy of chromosome 11p15.5 within the pancreas causes isolated hyperinsulinemic hypoglycemia. *Front Endocrinol* 2011;**2**:66