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An adult male patient with multiple adenomas and a hepatocellular carcinoma: mild Glycogen Storage Disease type Ia

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ABSTRACT

The development of hepatocellular adenomas and – more rarely – carcinoma in the liver of patients with Glycogen Storage Disease type Ia (GSDIa) is a well-known complication of the disease. The pathophysiology of adenoma and carcinoma development in these patients is, however, hitherto largely unknown and is thought to be related to the metabolic control of the patient and/or the type of mutations in the G6PC gene. We report here on a very illustrative case of adenoma and carcinoma formation in a previously undiagnosed 42 year old male GSDIa patient (enzymatically and genetically proven). He had 2 episodes of mild hypoglycaemia in childhood, never required formal treatment, showed normal growth, only mild lactate increases after prolonged starvation. He was a long-distance runner for most of his adult life, without the need for more than normal carbohydrate intake before/during exertion. To gain a better view on the type of adenoma formed in this patient, molecular studies were performed. We show here that in this patient with mild GSDIa without recurrent hypoglycaemic episodes adenoma and carcinoma formation still occurred and that malignant transformation of adenoma here is associated with CTNNB1 mutations and a typical mRNA profile of a beta-catenin activated lesion.

INTRODUCTION

Glycogen Storage Disease type Ia (GSDIa) or Von Gierke's disease (OMIM +232200) is an autosomal recessive disease caused by mutations in the G6PC gene encoding for glucose-6-phosphatase, an enzyme essential in the mobilisation of glucose from liver glycogen during fasting. Patients with this disease in general present in infancy, with ketotic hypoglycaemia, lactic acidosis, poor growth, hepatomegaly, hyperuricemia, hypertriglyceridemia, renal insufficiency. Traditionally, patients have to be kept in anabolic state by nocturnal nasogastric tube feeding and/or ingestion of uncooked corn starch to prevent life-threatening hypoglycaemia and hyperlactacidemia and to achieve normal development and growth, for life. In the second or third decade, a large majority of GSDIa patients develop hepatocellular adenomas, some of which evolve into hepatocellular carcinoma (HCC).[1,2] Because GSDIa is a rare disorder, the molecular subtype of the typical adenomas in these patients is largely unknown. On the other hand, to be able to distinguish adenomas associated with malignant degeneration from the adenomas that will not turn malignant would be very worthwhile, to allow adequate selection of patients with GSDIa and adenomas for tumor resection or liver transplantation.

Outside the context of GSDIa, it is becoming clear that adenomas that carry risk of malignant transformation are those carrying CTNNB1 mutations leading to an activation of ß-catenin, as opposed to the steatotic adenomas associated with HNF1A (Hepatocyte Nuclear factor 1 alpha) mutations and the inflammatory adenomas associated with IL6ST mutation, activating gp130.[3,4,5]

Up till now, mutation analysis and chromosomal studies of a total of 13 GSDIa-associated adenomas have been reported. Three of these adenomas were CTNNB1 mutated. [3,6,7] The only adenoma associated with an HCC, was CTNNB1 mutated.[7]

MATERIALS AND METHODS

Patient

The patient signed a written informed consent form, approved by the local Ehical Committee (Leuven University Hospitals, Belgium).

Pathology

The resection specimen was received freshly and 8 biopsies taken from the lesion were snap-frozen in liquid nitrogen-cooled isopentane and stored at -80° for molecular evaluation. Standard histopathological examination and histochemical stains were performed as described previously.[8] Immunohistochemistry for beta-catenin and glutamine synthetase were performed according to a recently described protocol.[9]

Molecular studies

Molecular studies (CTNNB1, HNF1A and IL6ST mutation analysis and real-time quantitative RT-PCR for LFABP1, UGT2B7, CRP, SAA, GLUL, GPR49, ANGPT1, ANGPT2, NTS, HAL) were performed as described, on all 8 biopsies.[3,5,7]

CASE DESCRIPTION

Patient history

A 42 year old male patient presented at the outpatient clinic, with abdominal pain and cramps for 2 weeks. Clinically, hard hepatomegaly was noted. On subsequent CT scan, a large (23cm by 12cm by 14cm) lesion was detected in the left liver lobe, showing an irregular margin, necrotic areas and calcifications. On MRI with contrast, multiple focal lesions mildly hyperintense on T2-weighted imaging, arterially vascularised and without late retention were noted, the largest lesion in segment 5/6 (3.5 cm), 5 (3.0 cm), 7 (1.7 cm), multiple similar lesions in segments 5, 6, 7 and 8 (all smaller than 2 cm). Based on the radiological appearance, preference for adenomas was accepted. Carcino-embryonic antigen and alpha-fetoprotein were not determined.

There were no metastases within or outside of the liver, so the lesion was resected. The patient underwent a laparoscopic left hepatectomy with preservation of the middle hepatic vein. This was done in a standard fashion using the surgical aspirator (CUSA Excel, Integra Life Science Ltd, IDA Business and Technology Park, Ireland) with minimal blood loss provided by an unilateral vascular inflow exclusion before starting the resection.The pathology report of the resection specimen, is described below.

As a child, between 1 and 2 years of age, he experienced two episodes of syncope with suspected hypoglycaemia, during viral illness. The treatment consisted of oral intake of glucose. Clinically at that time there was hepatomegaly. A diagnosis of glycogenosis was suggested but not pursued, followed-up or treated accordingly. On the contrary, the patient was started on

a diet for celiac disease, which he was still following at the age of 42. He did not have a history of recurrent hypoglycaemia, nocturnal feeding, lactic acidosis. He had a late puberty and growth spurt but excellent catch-up growth to a total length of 170 cm (weight 61kg). He never experienced any problems with fasting or exertion, he on the contrary is a long-distance runner, running marathons without apparent need for extra carbohydrates before or during exertion. After a 12h fast, lactate was 4.49 mM (0.4-2.0; in fed state 1.22 mM on one occasion, 2.01 mM on another), glucose after 12h fast was 64 mg/dl (55-100), triglycerides 328 mg/dl (<=180), cholesterol 151 mg/dl (<=190), uric acid 5.0 mg/dL (3.5-7.2). Glycaemia measured at home in the mornings, in fasting state, or when feeling weak or hungry, were consistently normal. Creatine kinase and liver tests were normal. Echocardiography was normal as well as glomerular filtration rate. There was no proteinuria, there was no history of kidney stones. During work-up for a gastroenteritis episode, 7 years prior to his presentation with the current lesion, ultrasound showed a ‘somewhat enlarged, homogeneous liver’ (no pictures stored for review). Family history is unremarkable. There is no consanguinity. The patient has no children, has one healthy sister without similar complaints (routine biochemistry and liver ultrasound normal), both parents are alive and well around the age of 70.

At 1 year follow-up after the resection, the patient has resumed his work and sports without restraint. A malignant lesion measuring 23 cm is well outside Milan criteria for transplantation. The patient has declined the theoretical option of transplantation. Four-monthly control MRI of the liver shows no recurrence of HCC and stable size of the remaining adenomas, chest CT

shows no lung metastases.

Pathology report of the resection specimen

The partial hepatectomy specimen measured 19 x 13 x 9 cm and contained a multinodular tumor with a maximal diameter of 18 cm. The tumor had a lightbrown color in some areas and a yellow to greenish aspect in other areas. Biopsies taken from the small rim of non-tumorous liver showed normal portal tracts and some fibrosis due to compression of the tumor. Very focally, there was some mixed steatosis. The periportal parenchyma contained numerous hepatocytes with a glycogenated nucleus (figure 1). Several hepatocytes also had a swollen, pale cytoplasm with wisps and small clumps of eosinophilic, giving the impression of so-called “plant-like” hepatocytes, which is suggestive of glycogen storage disease which is not of type 0 or 4 (figure 1).[11] Intense cytoplasmic hepatocytic positivity was not seen on the PAS-staining, most likely due to wash-out of glycogen during processing of the tissue.

The lesion consisted of several nodules of well differentiated hepatocellular carcinoma against a background of hepatocellular adenoma. The adenomatous areas consisted of sheets of non-steatotic hepatocytes organised as 1 to 2 cell thick plates separated by non-dilated sinusoids and traversed by some blood vessels. Reticulin surrounding these plates was preserved. Portal tracts, ductular reaction and inflammation were all absent. The nuclei of the hepatocytes showed mild atypia: they were slightly irregular and contained chromatin clumps and a distinctive nucleolus. Based on these morphological features (figure 2a), the adenoma was suspected to be of the beta-catenin activated subtype.[9] This was confirmed by

immunohistochemistry for glutamine synthetase which showed diffuse and strong staining of the adenomatous areas (figure 3a), while the surrounding, non-lesional liver only showed positivity in hepatocytes surrounding centrolobular veins (figure 3b). Nuclear expression of beta-catenin was observed in only very few hepatocytes in the adenoma. Positive staining of the hepatocytic cell membrane for beta-catenin served as an internal control.

The malignant nodules were sharply demarcated from the adenomatous areas and consisted of hepatocyte-like cells organised in trabecular and pseudoglandular structures. The trabecules were separated from each other by sinusoids and they were frequently more than 3 cells thick. The reticulin pattern was clearly lost. The nuclei were sometimes peripherally located within the cell and were slightly more atypical than in the adenomatous areas. The nucleo-cytoplasmic ratio was clearly increased. Bilirubin droplets were present in some of the malignant nodules. Based on these features (figure 2b), the diagnosis of well differentiated hepatocellular carcinoma arising in a beta-catenin activated hepatocellular adenoma was established. The malignant areas showed diffuse and strong positivity for glutamine synthetase (figure 3a). While all malignant hepatocytes showed positivity of the cell membrane for beta-catenin, there were very few positive nuclei.

Diagnostic work-up for suspected glycogenosis

Glycogen phosphorylase kinase enzymatic activity on fresh red blood cells was normal. On snap-frozen liver biopsy, glycogen was 4.8% of wet liver weight (3.53+/-0.45), enzymatic activity of phosphorylase and hexose

diphosphatase were normal, glucose-6-phosphatase activity was clearly reduced to 0.36 U/g (7.95 +/- 0.51).

Mutation analysis of the G6PC gene revealed compound heterozygosity for p.Arg170X (c.508C>T) in exon 4 and p.Ala192Val (c.575C>T) in exon 5. p.Arg170X has been described as a pathogenic mutation[10], while p.Ala192Val has not, but affects a phylogenetically highly conserved amino acid in a transmembrane domain of the protein and is thus predicted to be pathogenic.

Molecular studies of the HCC (8 biopsies)

Four random biopsies, taken from the large malignant lesion, showed the same mutation in CTNNB1: in-frame deletion Del7L-131L, while 4 other random biopsies showed another mutation in CTNNB1: in-frame deletion del21G-98M + ins21CC. No IL6ST or HNF1A mutations were found in any of the samples. We performed RT-PCR analysis testing the expression of 10 genes (LFABP1, UGT2B7, CRP, SAA, GLUL, GPR49, ANGPT1, ANGPT2, NTS, HAL) (Table 1). We found an mRNA expression profile typical of a ß-catenin activated adenoma in all samples, with overexpression of two genes targeted by ß-catenin (GLUL and GPR49) when compared to normal liver tissues (fold change T/N ranging from 3 to 374-fold, depending of the biopsies). Taken together, these results clearly demonstrate the presence of somatic CTNNB1 in-frame deletions activating ß-catenin in this lesion.

DISCUSSION

We report on a mild clinical presentation of GSDIa, where the diagnosis was missed early in life and the patient developed normally, without symptoms or complaints and without treatment or follow-up. Nevertheless, in adult age, the patient presented with multiple liver adenomas, one of which had developed into an HCC.

In the case presented here, the presence of hepatocytes with swollen and pale cytoplasm and with glycogenated nuclei in the tissue surrounding the resected hepatocellular carcinoma, together with a vague history of hypoglycaemia and hepatomegaly and an increased fasting lactate, set off the search for an underlying glycogenosis. The fact the patient has an enzymatically and genetically proven GSDIa and presented with adenomatosis and HCC in adult age, should prompt liver specialists and oncologists to look for GSDIa in patients presenting with adenomatosis and/or HCC, even when adult.

The presence of hepatocytes with swollen and pale cytoplasm is a well-known feature of GSD.[11] Although glycogenated nuclei in the periportal area are known to represent a disturbance of glycogen metabolism, as is seen in e.g. NASH[12], they have hitherto not been reported as a typical feature of GSD. Therefore, it is possible that they are related to the atypical clinical presentation of this patient. If not, the presence of glycogenated nuclei would be an additional and easy feature to prompt pathologists and clinicians to explore underlying glycogenoses.

The association of CTNNB1 mutations with malignant degeneration, is well known in sporadic liver adenoma.[3] Out of the 13 GSDIa-associated adenomas reported so far, 3 were CTNNB1 mutated.[3,6,7] Only one out of 13 was associated with an HCC and was CTNNB1 mutated. We describe another case here, where the resected malignant lesion carried different CTNNB1 mutations and an mRNA expression profile typical of a beta-catenin activation associated lesion (Table 1).

The discrepancy between the rarity of nuclear positivity for beta-catenin and the diffuse positivity for its down-stream target gene glutamine synthetase in the lesion described here, is remarkable. This has also been observed recently by Bioulac-Sage et al. in 3 CTNNB1 mutated sporadic adenomas.[9] This case report suggests that glutamine synthetase is a more sensitive marker than beta-catenin itself, when screening for possible beta-catenin mutation is performed by immunohistochemistry. This has to be studied and confirmed in a larger series of adenomas.

Finally, several types of CTNNB1 mutations were present in one malignant lesion here and all 8 random biopsies carried CTNNB1 mutations. This suggests that at least two adenomas with distinct mutation types have undergone confluence, either before or after malignant transformation, but that all lesions giving rise to the malignant lesion were CTNNB1 mutated.

In a setting of organ shortage and in view of the risks associated with liver transplantation, it would be useful to gain a better insight into the type of adenomas in GSDIa patients that are likely to undergo malignant transformation and therefore would strengthen the case for liver

transplantation.[13] By inference from what is know about sporadic adenomas, one would expect these to be the CTNNB1 mutated adenomas, i.e. glutamine synthetase positive or beta-catenin positive on immunohistochemistry. This is the second case of malignant transformation of a CTNNB1-mutated GSDIa-associated adenoma. If this association confirms itself in other patients, it would affect our treatment choices in these patients.

In conclusion, we describe a case of mild GSDIa, presenting with liver lesions in adult age. The HCC resected from this patient was CTNNB1 mutated, which confirms the premalignant nature of CTNNB1 mutated adenomas, also in GSDIa. Molecular studies for CTNNB1 mutations or routine immunohistochemistry for glutamine synthetase and beta-catenin, performed on punction biopsies taken from adenomas in GSDIa livers, could be useful to select patients with large and/or growing lesions for transplantation.

FIGURE LEGENDS

Figure 1: several hepatocytes show swollen, vacuolated nuclei. Hepatocytes with such glycogenated nuclei are mainly located in the area surrounding the portal tract (PT) (long arrows). The cytoplasm of hepatocytes frequently is swollen and is pale with small clumps of eosinophilic material. This “plant-like” aspect is most pronounced in the area indicated by the small arrow and enlarged in the inset. Original magnification x 400.

Figure 2: the adenomatous component of the multinodular lesion consist of trabecules of hepatocytes that show discrete nuclear atypia. The morphological features are consistent with a beta-catenin activated adenoma. Original magnification x 200 (left) and x 400 (right).

Figure 3: in contrast to the adenomatous part, the areas that transformed into well differentiated hepatocellular carcinoma show more distinct nuclear atypia and crowding due to an increased nucleo-cytoplasmic ratio and thickening of the trabecules. Original magnification x 400.

Figure 4: Both the adenomatous areas (A) as the parts that consist of malignant tumor (T) show diffuse and strong cytoplasmic positivity on the staining for glutamine synthetase, while surrounding non-lesional tissue serving as internal control only shows some staining in the area around the centrolobular vein (CV). Original magnification x 200.

TABLE LEGEND

Table 1: Real-time quantitative RT-PCR results on the 8 biopsies taken from the resected lesion

Nm = non mutated; M = mutated, AA = amino acid, H-HCA = adenoma inactivated for HNF1A, ßHCA = adenoma mutated for ß-catenin; IHCA = inflammatory adenoma.

*quantitative RT-PCR data expressed in 2^-DDCT, arbitrary unit, normal liver=1

**quantitative RT-PCR data, ratio of expression

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Table

Nodules ID	HNF1 Mutation	Mutation gp130	CTNNB1 mutation (nucleotide)	CTNNB1 mutation (AA change)	*GLUL**	*LGR5**	*FABP1**	*UGT2B7**	ANGPT1/ANGPT2**	*NTS/HAL***	*CRP**	*SAA2**
Biopsy 1	nm	nm	21_395del	7L-131Ldel	3.7	2.5	1	3.1	0.6	0.6	0.2	1.2
Biopsy 2	nm	nm	21_395del	7L-131Ldel	39.9	26.9	0.3	0.9	0.6	0.7	0.00	0.02
Biopsy 3	nm	nm	21_395del	7L-131Ldel	12.5	5.3	0.3	0.4	1.6	0.3	0,01	0.00
Biopsy 4	nm	nm	21_395del	7L-131Ldel	74.9	7.1	0.3	0.2	0.4	0.3	0.00	0.00
Biopsy 5	nm	nm	64-291del	21G-98Mdel+insCC	68	58	0.2	0.3	2.0	16.0	0.01	0.01
Biopsy 6	nm	nm	64-291del	21G-98Mdel+insCC	99.5	39.5	0.4	1	1.25	26	0.01	0.01
Biopsy 7	nm	nm	64-291del	21G-98Mdel+insCC	142.2	62.3	0.5	1.5	2.4	38	0.02	0.01
Biopsy 8	nm	nm	64-291del	21G-98Mdel+insCC	374.2	196.7	1.2	4.4	3	36	0.1	0.3
H-HCA (n=7)	M	nm	nm	nm	1.3	0.1	0.01	0.009	4.7	3.1	1.3	0.002
ßHCA (n=3)	nm	nm	M	M	96	56	0.6	1.7	4.3	13	0.2	0.58
IHCA gp130 mutated (n=6)	nm	M	nm	nm	2.7	2.1	0.85	3	2.3	2.6	36,00	35,00
Focal nodular hyperplasia (n=3)	Nm	Nm	nm	nm	18.4	21.8	0.3	2.3	12.1	386	0.1	0.2

We thank the reviewers and editors for their efforts to help us improve the manuscript, by detailed reading and commenting. We have fulfilled all requests for changes and additional information. Below, please find a point-by-point response to the reviewer's comments.

Highest regards,

David Cassiman

Reviewer #1:

Abstract
-How can you say metabolic control in this patient was not the cause of adenoma formation? The patient was not followed metabolically.

A: this was omitted.

Introduction
-"because hepatocellular adenomas in these patients rarely require surgical intervention" There are a number of publications that document surgical intervention in patients with GSD I and adenomas.

A: some centers prefer to adopt a wait-and-see strategy with careful radiological follow-up of the lesions, even if adenomas exceed the generally accepted limit of 5 cm in size. The section was omitted.

Case Description
-"As a child, between 1 and 2 years of age, he experienced two episodes of syncope with suspected hypoglycaemia, during viral illness. The treatment consisted of oral intake of glucose. Clinically at that time there was hepatomegaly. A diagnosis of glycogenosis was suggested but not proven. On the contrary, the patient was started on a diet for celiac disease, which he was still following at the age of 42." - We would argue that this was not a very mild case of GSD I. There were symptoms and signs of a GSD I diagnosis and "a diagnosis of glycogenosis was suggested" but because of lack of knowledge and work up, the diagnosis was missed.

A: this was changed accordingly: "A diagnosis of glycogenosis was suggested but not pursued, followed-up or treated accordingly."

-Did the patient have protienuria? What were the AFP and CEA values? Were these lab values done? Although we know they are not helpful in the adenoma setting, it is critical to know if in the HCC setting how they different from HCC in general population

A: aFP and CEA were not determined, there was no proteinuria. This was added.

-"During work-up for a gastroenteritis episode, 7 years prior to his presentation with the current lesion, ultrasound showed a somewhat enlarged, homogeneous liver." - Was this ultrasound re-reviewed after the diagnosis of HCC 7 years later? Is it possible there were undetected adenomas that could be identified when re-reviewed based on the current diagnosis? There is a growing body of evidence that imaging modalities for adenomas requires MRI with contrast, thus this point

needs to be highlighted in discussion and conclusions. Also, retrospective look may shed light on what may have been missed.

A: pictures were not available for review (ultrasound taken in emergency department setting, in a small rural hospital).

-"Four-monthly control MRI of the liver shows no recurrence of HCC and stable size of the remaining adenomas, chest CT shows no lung metastases." - Was this MRI with contrast? What were the size of the adenomas? Also, given the body of literature of recurrence of adenomas, the role of liver transplant in this setting needs discussion.

A: the requested data were added.

Discussion
-Again - we argue that this was not a "very mild" case of GSD I. There were clinical symptoms and signs in the patient from an early age. The patient was missed because of a lack of work up and instead treated for celiac disease. - not necessarily a "atypical clinical presentation" - maybe a milder clinical presentation. Current literature suggests there is a milder/less severe end of the GSD I phenotype. The patient had symptoms and in today's environment, the sypmtoms would have probably led to a diagnosis of GSD I. Also, the kidney involvenet- presence or absence of kidney stones, proteinuria and hypocitraturia are not mentione din teh clinical work up.

A: this was changed accordingly ('mild' instead of 'very mild'), the requested clinical data were added in the case description.

- Hepatomegaly was said to have resolved but was documented on the scan 7 years prior. "During work-up for a gastroenteritis episode, 7 years prior to his presentation with the current lesion, ultrasound showed a somewhat enlarged, homogeneous liver." However, the description of the ultrasound findings suggest an enlarged liver.

A: this passage was omitted from the discussion, as it was indeed based on non-reviewable data.

-Would like to see a mechanistic pathway for CTNNB1, activation of ß-catenin, etc.

A: length restrictions do not allow a detailed discussion of the literature beyond GSDIa, but further information is available in the cited literature.

- Discrepancy between the lack of nuclear positivity for beta-catenin and a weak positivity expression of its down-stream target gene glutamine synthetase in their case is unusual. Thus to test this hypothesis further studies need to be conducted in a larger cohort of GSD I patients with adenomas and also cases with HCC transformation of adenomas already. Also, can the authors comment on immunehitochemistry patterns that could help predict "adenomas at risk for transformation". In this clinical scenario pt had frank HCC , thus findings are not unexpected.

A: we indeed report this as 'remarkable' and refer to another study where this same discrepancy is reported. The fact this needs

confirmation in larger cohorts was added. The patterns that would classify adenomas as 'at risk' are also detailed in the discussion now.

- Point made by authors that they had seen 3 to 374 fold over expression of two down stream genes targeted by ß-catenin (GLUL and GPR49) in adenomas transformed HCC resected liver tissue as compared to normal liver tissues, it would be a good idea to show some proof of this statement showing RT-PCR/qPCR data obtained by the authors. Authors do not show any molecular or mRNA expression profile data obtained from this patient at all. It will be good to add some of that data to the paper.

A: new Table 1 contains all the available data.

Overall, this paper has approached the characterization of the HCC found in a patient using an immuno-histochemistry and other histopathology approaches to get a retrospective diagnosis of GSD Ia in this patient. What the authors fail to mention is clinically the patient could have been diagnosed with GSD Ia, with appropriate FU.

A: this is mentioned now, in the case description (see above, also).

What did immunohistochemistry offer that routine histology did not, in terms of a diagnosis of HCC, Are there differences in HCC in GSD I and general population on immunohistochemistry? This needs to be clearly stated. Also what is the ease of doing these studies in a general setting. Are they routinely done in HCC?

A: beta catenin and glutamine synthase staining of resected or biopsied adenomas are becoming common practice, since they allow easy classification of the lesion and therefore determine the follow-up and treatment. Immunohistochemical staining is a routine pathology lab technique.

Reviewer #2:

Mutation analysis of the G6PC gene revealed a previously described mutation and a novel one whose pathogenicity has not been proved. It would be useful to know whether this mutation has been searched in controls, so that a polymorphism may be ruled out.

A: The fitting combination of clinical picture, routine blood work-up and blood testing after fasting, the enzymology on liver tissue, the liver pathology and the finding of one known mutation together with a mutation that is suspected to be pathogenic was considered sufficient to make the diagnosis. We did not check the mutation/polymorphism in healthy controls.

In the discussion, mild GSDIa have been prviously reported with prolonged fasting tolerance and this point should be corrected.

A: was corrected

Finally, even though the paper does not focus on these points, it would be interesting to know whether hepatectomy was difficult or not (bleeding in particular),

A: this information was added in the case description: "The patient underwent a laparoscopic left hepatectomy with preservation of the middle hepatic vein. This was done in a standard fashion using the surgical aspirator (CUSA Excel, Integra Life Science Ltd, IDA Business and Technology Park, Ireland) with minimal blood loss provided by an unilateral vascular inflow exclusion before starting the resection."

and to know why the authors have not discussed liver transplantation in this patient, as it has been recommended when hepatocarcinoma occurs.

A: this passage was added to the case description "A malignant lesion measuring 23 cm is well outside Milan criteria for transplantation. The patient has declined the theoretical option of transplantation."