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CASE REPORT

Unclassified hepatocellular adenoma expressing ASS1 associated with inflammatory hepatocellular adenomas

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KEYWORDS
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Summary Three liver nodules were fortuitously discovered in a 30-year-old obese woman during a gynecological workup and resected. Two nodules (6 and 1.5 cm) with histological characteristics of inflammatory hepatocellular adenoma (HCA) were C reactive protein positive with normal expression of glutamine synthetase. The third 6 cm nodule had all the characteristics of an Unclassified HCA with an overexpression of Argininosuccinate Synthase 1 (ASS1) in the tumor compared to the non-tumoral liver. The non-tumoral liver was highly steatotic. Upon MRI review, two HCAs were identified as inflammatory HCAs based on specific criteria. The third HCA was different from the other two with the presence of peculiar intratumoral fluid cavities. This first report on the association between unclassified HCA expressing ASS1 and inflammatory HCA reinforces the concept that common factors are implicated in HCA subtypes genesis. ASS1 is an interesting immuno-marker to identify among unclassified HCA a subgroup with a high risk of bleeding. ASS1 overexpression decreases sharply the number of ''true'' unclassified HCA.

Background

Hepatocellular adenomas (HCA) are classified into different immuno-subtypes [1,2]. Compared to the non-tumoral liver, different immuno-markers expression corresponds to different molecular subtypes, basis of the HCA classification [3]:

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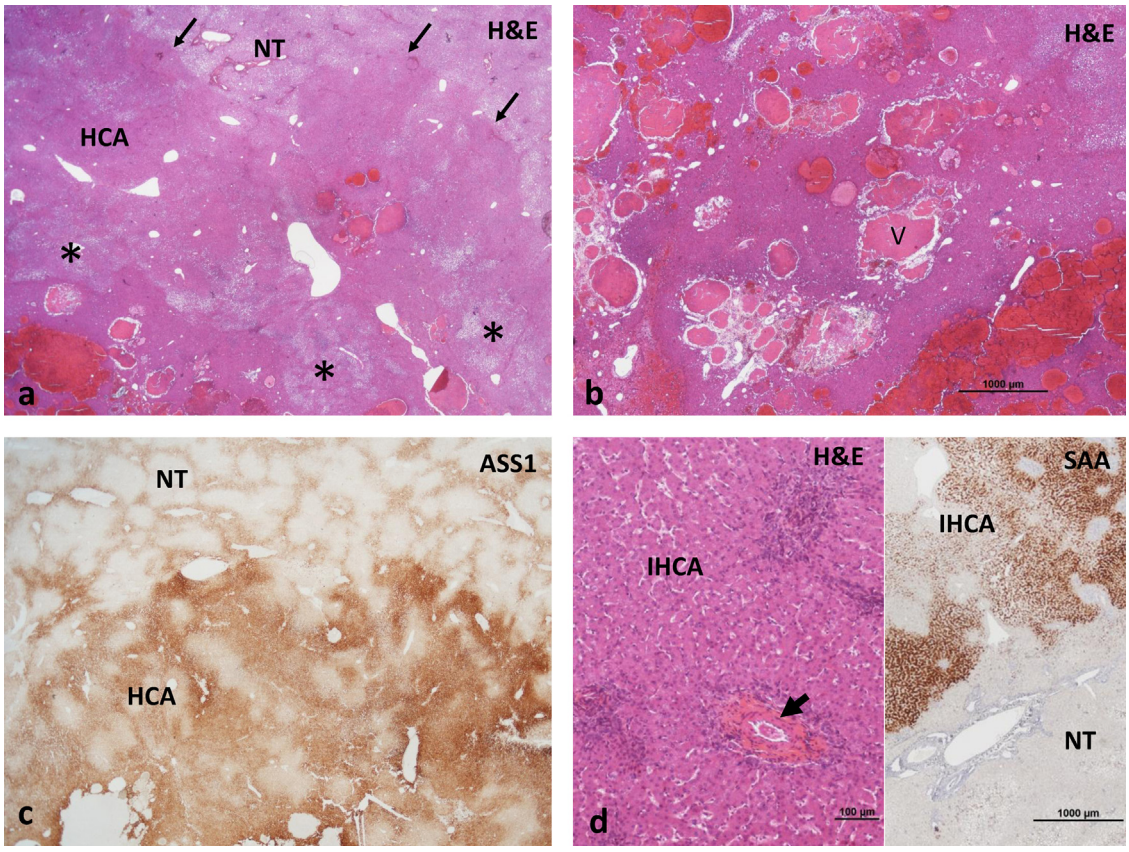

Figure 1 Pathology nodule N1 (UHCA expressing ASS1: a, b, c) ; nodule N2 (d: IHCA); a, b: numerous vacuoles (V), filled of serosities are dispersed within the HCA; arrows indicate the limits between HCA and non tumoral liver (NT), with steatotic areas in the HCA (asterisk). c: overexpression of ASS1 in the HCA in comparison with NT; d: typical aspect of IHCA; inflammation, thick artery (thick arrow) (left); strong expression of SAA contrasting with NT (right).

- *HNF1A* mutated (inactivated) HCA (H-HCA) with LFABP negative expression;
- inflammatory HCA (IHCA) corresponding to different mutated (activated) genes with CRP/SAA positive expression;
- *CTNNB1* mutated HCA coding for β-catenin HCA (b-HCA) /b-IHCA with different types of abnormal glutamine synthetase staining, depending to the level of β-catenin pathway activation [4,5], and;
- sonic Hedgehog HCA (shHCA), defined by *INHBE- GLI1* fusion with expression of Prostaglandin D synthase (PTGDS) [3,6]. Recently using a proteomic approach [7]: first, regarding protein expression, among unclassified HCA (UHCA) the great majority appeared as a homogeneous subgroup, which was different from the other HCA subtypes; second, we observed that the arginine biosynthesis pathway, including Argininosuccinate Synthase (ASS1) and Argininosuccinate Lyase (ASL), was significantly up-regulated in UHCA when compared to non-tumoral tissue. These data were confirmed by immuno-histochemistry (IHC), qPCR, and western blotting analyses in a larger cohort (7).

HCA can be solitary or multiple and in the latter case, they are usually of the same subtype. Here we report, for the first time, an UHCA expressing ASS1 associated with IHCA.

Case report

During a gynecological examination, liver nodules were discovered in a 30-year-old woman with a high body mass index (33.3) and an elevated GGT (4 N). The patient was taking oral contraceptives (Adepal) for 20 years; she was not diabetic and did not have arterial hypertension.

On CT scan and MRI, three nodules were discovered: N1, 6 cm (V VI VII); N2, 6 cm (IV); N3, 1.5 cm (VI) and surgery was performed in 2003. Follow-up was uneventful. Nodules were identified as HCAs. The non-tumoral liver was highly steatotic (60%).

Years later, nodules were reviewed using IHC to classify HCAs [1]. Liver pathology (Fig1) showed typical features of IHCA for N2 and N3, expressing CRP and SAA, without abnormal GS staining. N1 did not show any IHCA features by standard and immuno- histology; it was reviewed in 2017. This nodule showed the H&E characteristics of an unclassified HCA expressing ASS1, a newly identified HCA subtype [7]. The bland, well differentiated hepatocellular proliferation was intermingled with multiple cavities containing red blood cells, blood degradation products or serosities (Fig. 1a, b); there was no inflammation. The border with the non-tumoral liver was not well defined due to the presence of some steatotic hepatocytes inside the nodule as in the non-tumoral liver. All IHC markers of

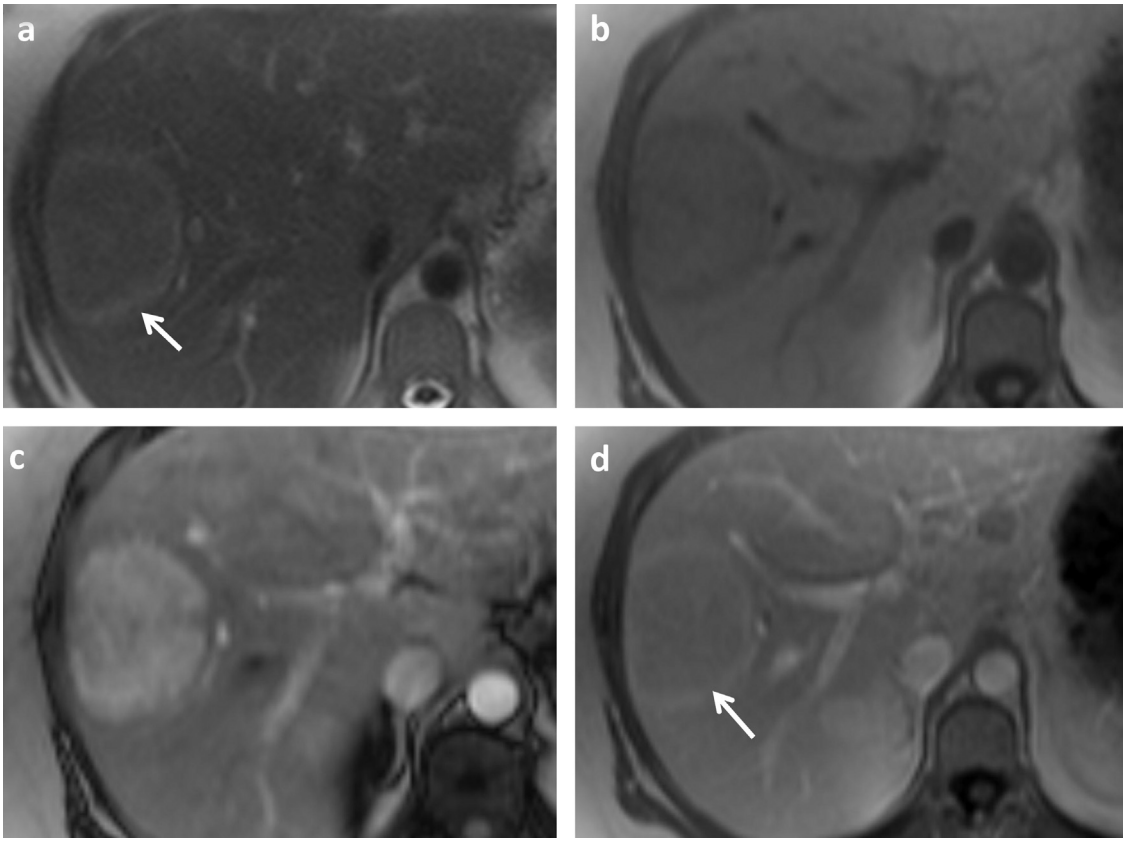

Figure 2 MRI nodule N2 (IHCA); a: T2 weighted imaging : the lesion presents T2 hyperintense signal band in periphery (black arrow) with center isointense to surrounding liver (typical atoll sign); b: T1 weighted imaging : the lesion appears isointense to surrounding liver whereas the peripheral band is slightly hypo intense; c: Arterial phase shows a strong and homogenous arterial enhancement after gadolinium intravenous administration; d: at portal venous phase, the center of the lesion is isointense to surrounding liver whereas the peripheral band remains hyperintense correponding to sinusoidal distension (black arrow).

HCA were negative except ASS1 which was overexpressed compared to the non-tumoral liver (Fig. 1c).

MRI performed in 2003 was reviewed in 2017. N2 and N3 were typical IHCA, as previously described [8] (Fig. 2) and expressed ASS1 although with less intensity than N1, at least in the larger IHCA nodule. In N1, the imaging aspect was completely different showing an isointense lesion with several central cavities with strong hypersignal in T2 weighted imaging and hyposignal in T1. At the arterial phase, there was a strong enhancement of the lesion except in the central ''fluid'' cavities which were in hyposignal. At the portal venous phase, the ''fluid'' cavities remained in hyposignal whereas the rest of the lesion was in iso signal to the surrounding liver parenchyma (Fig. 3). Interestingly, we observed same MRI features in other UHCA expressing ASS1 cases of our own series.

Discussion

UHCA expressing ASS1 is a recently described specific HCA subtype [7] characterized by frequent hemorrhage of different types: subcapsular or intratumoral hematoma, peritoneal bleeding with often severe clinical manifestations. It is usual to observe, by standard histology, well circumscribed cavities filled with blood degradation products, empty vacuoles or filled with pale pink to red serosities, some fibrotic bands often hemorrhagic, inside the tumor or delineating the tumor from the non-tumoral tissue. The other characteristics are the presence of large areas of clear hepatocytes separated by strands of packed smaller cells often located along arterial axis. They also present cytological abnormalities with frequent picnotic nuclei related to ischemic damage. Occasional sinusoidal dilatation, rupture of the sinusoidal wall, congestion and peliosis areas can also be observed. It is remarkable that the tumor exhibited several areas of bleeding sequellae whereas the patient did not present acute clinical manifestations.

Interestingly, we observed that the 2 other nodules — which were typical IHCA — were not hemorrhagic, but overexpressed ASS1. This ASS1 positivity has already been observed occasionally in other HCA subtypes, particularly IHCA [7] but has not yet received a satisfactory explanation; it probably highlights common etiologic factors. Hence, ASS1 not being a specific marker, it is recommended to exclude all known HCA subtypes by performing appropriate assays for HCA markers before identifying the subtype UHCA expressing ASS1.

In this case report, we did not perform tests for PTGDS, a marker of shHCA [3—6] that shares UHCA expressing ASS1 characteristics, because the staining is not sensitive enough

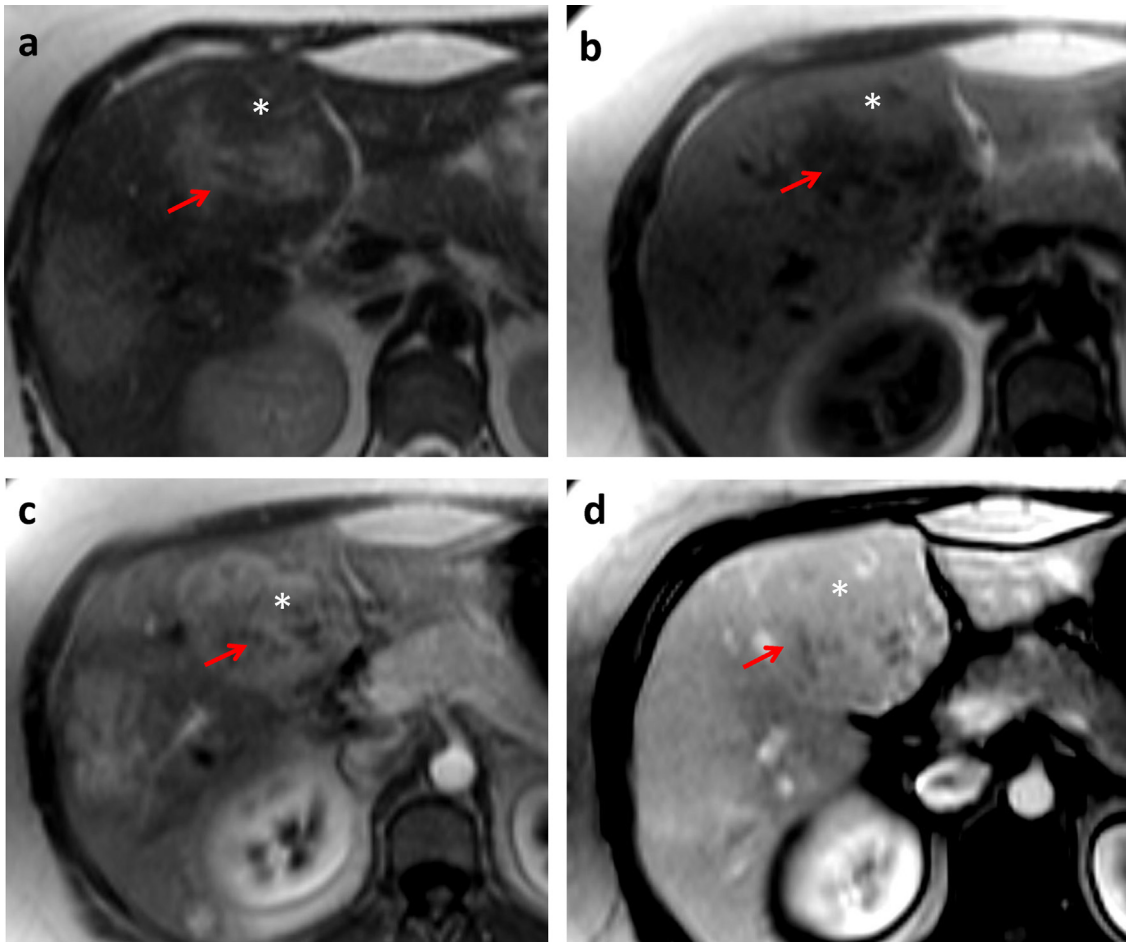

Figure 3 MRI nodule N1 (ASS1 + HCA); a: T2 weighted imaging : the lesion(*) is isointense to surrounding liver with focal central ''confluent'' cavities with strong hypersignal (red arrow)); b: T1 weighted imaging : the lesion (*) is isointense to surrounding liver with focal central cavities in hyposignal (red arrow); c: Arterial phase: strong arterial heterogenous enhancement of the lesion (*) with no enhancement of the central 'fluid cavities '(red arrow); d: Portal venous phase : the lesion (*) remained heterogenous in isosignal to surrounding liver with central fluid cavities in hyposignal (red arrow).

[9] to differentiate them. In our experience, few shHCA cases express PTGDS. In the absence of data from other liver centers working on UHCA claiming that PTGDS is a specific and sensitive marker to identify this new shHCA subgroup identified by molecular analysis, we still used our proteomic approach and ASS1 immunostaining. As in shHCA, we confirmed that UHCA expressing ASS1 was a subgroup of major interest associated with bleeding. A multicentric validation in a larger set of tumors is required to compare ASS1 and PTGDS IHC for the diagnosis of shHCA [6].

In a recent large study, Nault et al. [3] have shown that in the vast majority of cases with multiple HCAs, the nodules were of the same subtype. In our series of 217 resected HCA at the University Hospital in Bordeaux, association of two different HCA subtypes is rare and has been reported in women only [10] but not so between IHCA and UHCA expressing ASS1.

The highlight of this case report is the identification, through MRI, of the two different HCA subtypes. Current imaging techniques allow the identification of typical IHCAs [8]. UHCA expressing ASS1 was not identified as such but the presence of peculiar fluid cavities inside the lesion corresponding to the cavities with serosities observed under the microscope highlights the characteristic feature of this subtype. Among the 14 UHCA expressing ASS1 reviewed with exploitable MRI, six presented a bleeding with recent hemorrhagic remodeling of the lesion > 75% due to acute abdominal pain. In the 8 remaining cases, excluding the context of recent and symptomatic bleeding, more than 60% (5/8 cases) presented with these fluid cavities in MRI. More studies are needed to confirm the relevance of this finding.

In conclusion UHCA expressing ASS1 can be associated with other HCA subtypes which highlights probable common etiologic factors.

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Disclosure of interest

The authors declare that they have no competing interest.

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