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Clinical phenotype of adult-onset systemic histiocytosis harboring *BRAF* in-frame deletions

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L-group histiocytoses (Erdheim-Chester disease (ECD) and Langerhans-cell histiocytosis (LCH)) are multi-system diseases characterized by histiocytes infiltration in several organs [1–3]. In these diseases, histiocytes frequently display activating somatic mutations of intracellular signaling pathway protein kinases (mostly the MAPKinase pathway) [1–3]. Many of these mutations seem to be associated with a specific phenotype: $BRAF^{V600E}$ mutated ECD patients have more frequently cardiac and retroperitoneal manifestations[4], *MAP2K1* mutated ECD patients may exhibit overt Rosai-Dorfman Disease (RDD) component[5], and *ALK*-mutated patients have a high prevalence of neurological manifestations[6]. Therefore, we suspect that each specific mutation or mutation type could be associated with a specific clinical phenotype of histiocytosis. Our objective was to describe characteristics of patients with histiocytosis and in-frame deletion within exon 12 of *BRAF* ($BRAF^{\Delta\beta3\text{-}\alpha C}$).

Inclusion criteria were: 1) Diagnosis of histiocytosis confirmed by central review according to most recent published guidelines [3,7], 2) presence of $BRAF^{\Delta\beta3\text{-}\alpha C}$, and 3) clinical data available. Patients were retrieved from the files of pathology laboratory in Ambroise-Paré Hospital (Boulogne, France). Clinical, biological and morphological data were retrieved, as well as treatment received and follow-up.

DNA was extracted as previously described [8]. Since 2020, DNA extraction has been automated on a Maxwell® RSC Instrument (Promega, France), with extraction performed according to the supplier's recommendations. For formalin-fixed paraffin-embedded (FFPE) and frozen biological materials, the Maxwell® RSC DNA FFPE Kit and Maxwell® RSC Tissue DNA Kit were used, respectively. The DNA-Seq Next Generation Sequencing (NGS) panel included almost 60 genes covering hot spots or all exons previously reported to be mutated in histiocytoses and genes involved in the MAP-kinase pathway and myeloid

neoplasia. The sequencing data were analyzed depending on the applied technique. Mutations detected by DNA sequencing were interpreted according to standards and guidelines as previously described[9].

We identified patients with *BRAF*$^{\Delta\beta3\text{-}\alpha C}$ and contacted the centers for clinical, morphological, and biological data.

This study was conducted in accordance with the Declaration of Helsinki. Patients provided written informed consent (clinical trial registration NCT04437381 [Molecular Targets for the Treatment of Histiocytosis HISTIO-TARGET]).

In 429 patients with L-group histiocytosis cohort, 189 had a *BRAF*^{V600E} mutation (46.2%), 25 had a *BRAF*$^{\Delta\beta3\text{-}\alpha C}$. (5,8%) and 9 another *BRAF* mutation (2,1%). Among patients with *BRAF*$^{\Delta\beta3\text{-}\alpha C}$, data were available for 20 of them. Patients and mutations characteristics are described in **Table 1 and Figure 1**. Most patients (n=18) had LCH, and two had an ECD, one pure and the other mixed with LCH. Median age at diagnosis was 50.5 years (IQR 34-78). The most frequent manifestations were hepatic (n=9, 45%) and vulvar (8/11 female gender patients, 73%). Other localization were cystic interstitial lung disease (n=7), lytic bone lesions (n=8), classical cutaneous manifestations (n=8), diabetes insipidus (n=8), panhypopituitarism (n=3), pachymeningitis (n=2), long bone osteosclerosis (n=1), perirenal infiltration (n=1), salivary gland infiltration (n=1) and digestive track infiltration (n=1). Among patients with LCH, 2 patients had a single-system pulmonary disease, one patient a single-system liver disease, one patient a single-system multifocal bone disease, and all the others had multi-system manifestations.

Hepatic manifestation was sclerosing cholangitis in all patients, and 5/6 patients had histiocytic infiltration in liver biopsy. All patients with sclerosing cholangitis had biological cholestasis, elevated aminotransferases, and hyperbilirubinemia. No patient had cirrhosis.

Hepatic MRI, when performed, always showed cholangitis (6/6). PET-scan showed liver abnormalities in 4/7 patients (heterogenous liver uptake or uptake in biliary ducts). Six patients had additional mutations in tissue biopsy, including *DNMT3A* (n=4), *TET2* (n=2), *ASXL1* (n=1) and *PGDFRA* (n=1). Among 5 patients who had a bone marrow aspiration, 4 of them had additional mutations, including *DNMT3A* (n=2), *TET2* (n=2), *STAG1* (n=1), *PPM1D* (n=1) and *RAD21* (n=1).

First line treatments included vinblastine (n=6), cytarabine (n=1), methotrexate (n=1), cladribine (n=3), lenalinomide (n=1) and cobimetinib (n=1), with various responses depending on the clinical manifestation. Nine patients did not receive any specific treatment for histiocytosis. Four patients with cholangitis received ursodeoxychlic acid without significant improvement. Patients with cholangitis also received vinblastine (n=3 with one disease progression, one stable disease, and one partial remission), cladribine (n=1 with stable disease), cytarabine (n=1 with stable disease) and lenalimode (n=1 with stable disease). Two patients received cobimetinib, that resulted in partial remission in both patients (PERCIST criteria) at 6 months, while liver function testes and bili-MRI remained stable in one patient (**Figure 2**). One patient had a liver transplant, with no further relapse. After a median follow-up of 47 (IQR 13-315) months, one patient had died from coronary heart disease.

Clinical manifestations of L-group histiocytosis may vary from single-organ benign disease to multi-organ life-threatening neoplasm[2,3]. To date, the cause of the variety of clinical manifestations in these diseases is unknown, and the type of mutation involved could play a role in the clinical phenotype[4–6].

Our study is the first to describe the clinical phenotype of histiocytosis patients with a $BRAF^{\Delta\beta3\text{-}\alpha C}$, and showed a high frequency of sclerosing cholangitis and vulvar manifestations,

which are typical LCH manifestations but usually rarely observed. In previous published cohorts of adults, liver manifestations are described in 10-15% of LCH cases [10], and a study of 14 pediatric patients with LCH and liver involvement showed a 100% prevalence of $BRAF^{V600E}$ mutation[11].Vulvar manifestations have only been described in some cases series[12]. They can present as erythematous plaques, eczema, ulcer or polypoid appearance, which are non-specific and sometimes it can mimic many other diseases, such as squamous cell carcinoma, malignant melanoma, herpes or some inflammatory reaction[12].

$BRAF^{\Delta\beta3\text{-}\alpha C}$ were described in pancreatic, lung, ovarian, thyroid cancers and melanoma[13], and also occur in histiocytoses. These oncogenic deletions are predicted to shorten the β 3/ α C-helix loop, which could favors dimer formation. They are resistant to the BRAF monomer inhibitors, such as vemurafenib but sensitive in vitro to BRAF dimer inhibitors and MEK inhibitors[14]. So far only two patients with histiocytosis harboring $BRAF^{\Delta\beta3\text{-}\alpha C}$ have been reported with targeted therapy, and both had complete remission with either trametinib[15] or cobimetinib[16]. Two patients of our series were treated with MEK inhibitors, with partial remission on PERCIST criteria, but no significant improvement in liver function tests and MRI cholangiopancreatography (**Figure 2**). Based on the low response rate of standard chemotherapy in liver locations, those patients may require first line treatment with MEK-inhibitors.

To conclude, *BRAF*-deletions mutations in histiocytoses seem to be associated with a specific LCH pattern with high prevalence of hepatic and vulvar involvements. These manifestations should be carefully screened in these patients. These results also comfort the hypothesis that each specific mutation in histiocytosis correlates with a specific clinical phenotype.

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Table 1. Characteristics of L-group histiocytosis patients with *BRAF* in-frame deletion

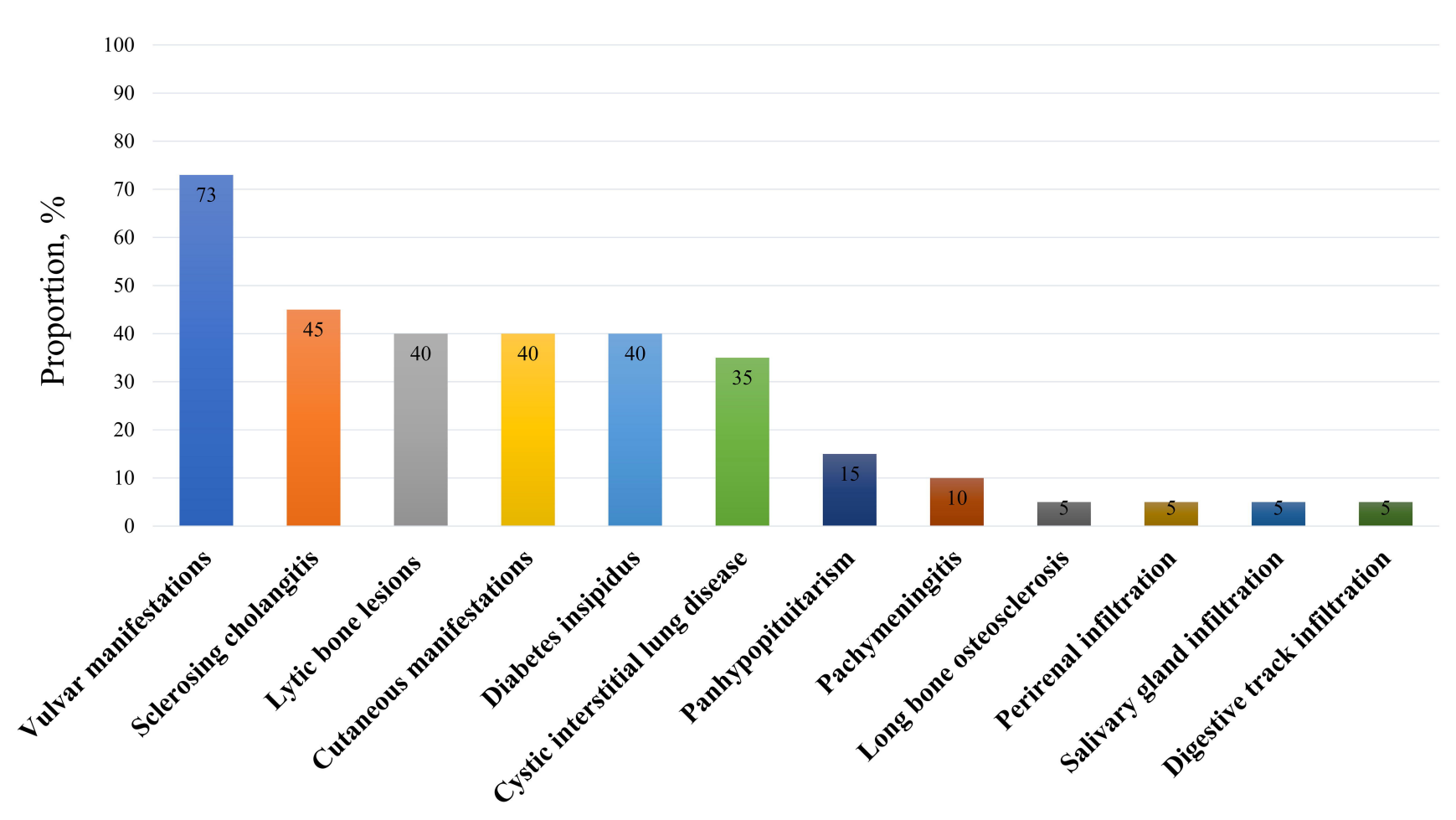
Patient	Gender	Age at diagnosis (years)	*BRAF* mutation	Histiocytosis	Clinical manifestations	Treatment & outcome	Follow-up duration (months)
#1	M	78	c.1459_1473del	LCH, ECD	Sclerosing cholangitis, cystic interstitial lung disease, lytic bone lesions	None (Death from coronary heart disease)	14
#2	M	74	c.1457_1471del	LCH	Sclerosing cholangitis	UDCA (stable disease)	11
#3	M	66	c.1457_1471del	LCH	Cystic interstitial lung disease	None	29
#4	F	59	c.1457_1471del	LCH	Vulvar manifestations, cutaneous manifestations, lytic bone lesions, diabetes insipidus	Vinblastine (disease progression), cladribine (disease progression)	98
#5	F	65	c.1457_1471del	LCH	Sclerosing cholangitis, vulvar manifestations, cutaneous manifestations, diabetes insipidus	Liver Transplantation (remission)	55
#6	M	56	c.1471_1476del	ECD	Long bone osteosclerosis, pachymeningitis, perirenal infiltration	Cobimetinib (partial remission)	120
#7	F	61	c.1457_1471del	LCH	Sclerosing cholangitis, vulvar manifestations, lytic bone lesions, diabetes insipidus	UDCA (stable disease) Vinblastine (disease progression), cobimetinib (partial remission)	43
#8	F	33	c.1457_1471del	LCH	Vulvar manifestations, cutaneous manifestations, cystic interstitiel lung disease, lytic bone lesions, diabetes insipidus, panhypopituitarism	Methotrexate (stable disease)	315
#9	F	56	c.1457_1471del	LCH	Sclerosing cholangitis, cystic interstitial lung disease	None	0
#10	F	45	c.1457_1471del	LCH	Vulvar manifestations, cutaneous manifestations, lytic bone lesions, diabetes insipidus, pachymeningitis	None	89
#11	M	41	c.1457_1471del	LCH	Sclerosing cholangitis, cutaneous manifestations, salivary glands infiltration	Cytarabine (skin improvement, cholangitis stability), cladribine (skin improvement, cholangitis stability), lenalinomide (skin improvement, cholangitis stability)	44
#12	M	34	c.1459_1473del	LCH	Cystic interstitial lung disease	None	3
#13	F	21	c.1457_1471del	LCH	Vulvar manifestations, cutaneous manifestations, diabetes insipidus, panhypopituitarism	Cladribine (complete remission)	148
#14	M	23	c.1457_1471del	LCH	Sclerosing cholangitis, digestive track infiltration	UDCA (stable disease), Vinblastine (partial remission)	62
#15	M	17	c.1458_1472del	LCH	Lytic bone lesions	None	42
#16	F	39	c.1458_1472del	LCH	Cystic interstitial lung disease	Vinblastine (partial remission)	2
#17	F	58	c.1457_1471del	LCH	Vulvar manifestations, panhypopituitarism	None	10
#18	F	38	c.1457_1471del	LCH	Sclerosing cholangitis, cystic interstitial lung disease	None	50
#19	F	73	c.1457_1471del	LCH	Sclerosing cholangitis, cystic interstitial lung disease, vulvar manifestations, cutaneous manifestations, diabetes insipidus	UDCA (stable disease), Vinblastine (stable disease)	77
#20	M	11	c.1457_1471del	LCH	Cystic interstitial lung disease, peri-anal manifestations, diabetes insipidus, lytic bone lesions	Vinblastine (remission and relapse)	146

M: Male; F: Female; LCH: Langherans Cell Histiocytosis; ECD: Erdheim-Chester Disease UDCA: Ursodeoxycholic acid

Figure 1. Proportion of clinical manifestations in patients with histiocytosis and *BRAF* in-frame deletions

Figure 2. Sclerosing cholangitis in Langerhans cell histiocytosis patients with *BRAF* in-frame deletion

A. Intense and diffuse hypermetabolism of the intrahepatic biliary ducts (SUVmax 9.5) on FDG PET-CT before cobimetinib onset **B.** Partial regression of intense and diffuse hypermetabolism of the intrahepatic biliary ducts (SUVmax 5.9) on FDG PET-CT six months after cobimetinib onset **C**-**D**. MRI cholangiopancreatography performed at onset (**C**) and after six months (**D**) of cobimetinib treatment. The main bile duct (white arrow) is normal, as is the main pancreatic duct ("empty arrow"). Numerous peripheral bile ducts appear multifocally dilated and suspended in the right (empty arrowhead) or left (arrowhead) liver. The successive examinations showed no change in the number, distribution or dilatation of intrahepatic bile ducts **E-F** Large portal tract with destructive infiltration of biliary duct by numerous mononucleated histiocytes (x50) (**E**) expressing CD1a (**F**).


Proportion, %
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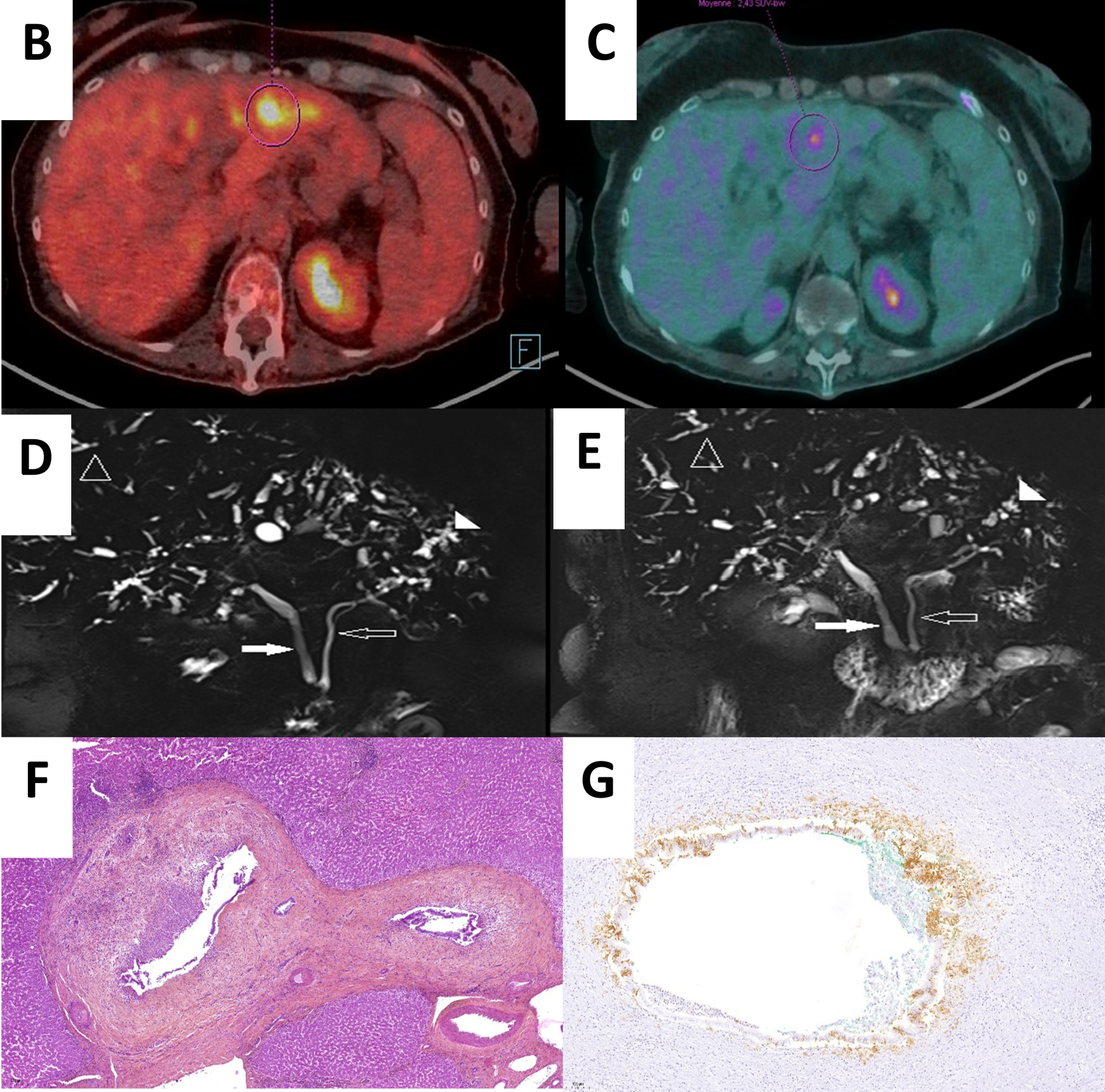
Vulvar manifestations
Sclerosing cholangitis
Lytic bone lesions
Cutaneous manifestations
Diabetes insipidus
Cystic interstitial lung disease
Panhypopituitarism
Pachymeningitis
Long bone osteosclerosis
Perirenal infiltration
Salivary gland infiltration
Digestive track infiltration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C
Moyenne : 2,43 SUV-bw
D
E
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G