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Original Article

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Central nervous system candidiasis beyond neonates: Lessons from a nationwide study

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Abstract

Though candidiasis is the most frequent invasive fungal infection, *Candida* spp. central nervous system (CNS) infections are rare but severe. To further describe clinico-patho-radiological presentations of this entity, we report a retrospective study from January 2005 to December 2018 including patients aged ≥ 28 days with proven or probable CNS candidiasis in France. Twenty-four patients were included. Seventeen patients (70%) had CNS localization secondary to disseminated candidiasis (10 with hematologic malignancies [HM]; the seven other patients had infective endocarditis [IE]). Among patients with HM, seven previously had lumbar puncture for intrathecal chemotherapy, the three others had IE. Among patients with disseminated infection, magnetic resonance imaging (MRI) evidenced meningitis (17%), micro-abscesses (58%), or vascular complications (67%). Seven patients (30%) had isolated CNS involvement related to neurosurgery ($n = 2$), CARD9 deficiency ($n = 2$), intravenous drug use, diabetes mellitus, or no identified predisposing condition ($n = 1$ each). All evaluated patients with isolated CNS involvement had meningitis on cerebrospinal fluid (CSF) and intracranial hypertension. For the latter patients, MRI evidenced meningitis (71%) or abscesses (57%). Among all patients, cerebrospinal fluid (CSF) culture grew *Candida* spp. in 31% of cases. CSF βDGlucan or mannan Ag were positive in respectively 86% and 80% of cases. Mortality attributed to CNS candidiasis was 42%: 53% in case of disseminated infection (70% for HM) and 14% in case of localized infection. CNS candidiasis are isolated or occur during disseminated infection in patients with HM and lumbar puncture for intrathecal chemotherapy or during IE. Clinical, radiological finding and outcome highly vary according to CNS localized versus disseminated candidiasis.

Lay Summary

Candida is a yeast and is the most common cause of fungal infections worldwide.
Candida central nervous system (CNS) infections are rare, severe, and poorly described. We report a retrospective study from January 2005 to December 2018 including patients aged ≥ 28 days with proven or probable CNS candidiasis in France. Twenty-four patients were included (14 men, median age 51 years). Seventeen patients had CNS localization secondary to disseminated candidiasis from blood to CNS (10 with hematologic malignancies [HM], the seven other patients had infective endocarditis [IE]). Seven patients had isolated CNS involvement related to neurosurgery ($n = 2$), CARD9 deficiency ($n = 2$), intravenous drug use ($n = 1$), diabetes mellitus ($n = 1$), or no identified risk factor ($n = 1$).
During *Candida* CNS infections, brain lesions were meningitis abscesses or vascular complications. Cerebrospinal fluid (CSF) culture grew *Candida* spp. in 31% of cases. Forty-two percent of patients died from infection: 53% in case of disseminated infection (70% for HM) and 14% in case of localized infection.

Key words: *Candida*, central nervous system fungal infections, hematologic neoplasms, endocarditis, inherited CARD9 deficiency.

Introduction

Invasive candidiasis is the most frequent cause of invasive fungal infections in France[1] and is associated with a persistent high mortality.[2] Despite high frequency of central nervous system (CNS) candidiasis reported in old autopsy studies with presence of *Candida* spp. in 49% of the cases of cerebral mycosis[3] and CNS involvement in 48% of candidiasis,[4] dissemination to the CNS is nowadays rarely reported except in neonates due to blood brain barrier immaturity.[5] CNS candidiasis is mainly reported in patients with hematological malignancy (HM), organ transplant, intravenous drug use (IVDU), diabetes mellitus, or human immunodeficiency virus (HIV).[6,7] CNS *Candida* spp. infections are also described following neurosurgery[8] or in patients with primary immunodeficiency such as CARD9 deficiency.[5,9] *Candida* spp. infective endocarditis (IE) are complicated with CNS embolic complications in 12 to 22% of cases.[7,10,11] Underlying conditions, clinical, pathological, and radiological presentations, diagnostic tools, management and prognosis of CNS candidiasis are poorly described. We therefore conducted a retrospective study in France to better describe underlying conditions, clinico-patho-radiological presentations, diagnostic tools, and management of CNS candidiasis outside neonatalogy.

Methods

We performed a retrospective nationwide study of CNS candidiasis in France from January 2005 to December 2018. French mycologists and infectious disease clinicians were informed about the study by their scientific societies: the French Medical Mycology Society and the Infectious Diseases French Society. Cases were identified in each institution using screening of local mycological databases or clinicians declarations. Anonymous data were collected in centers, sent and validated by investigators. Six patients with *Candida* spp. endocarditis were also previously reported.[7,11] Data were collected with a standardized case report form. All 15 hospitals participating to this study were tertiary care centers. Eligible patients were aged ≥ 28 days and met criteria for proven or probable CNS candidiasis according to the 2008 EORTC/MSG definitions.[12] Proven CNS candidiasis diagnosis required *Candida* growth from a normally sterile sample (cerebrospinal fluid [CSF], brain biopsy, or blood) and clinical and/or radiological CNS abnormality consistent with an infectious disease process with the lack of alternative diagnosis. Probable CNS candidiasis required host factor, clinical features (focal lesions on imaging or meningeal enhancement on magnetic resonance imaging (MRI)), and mycological evidence ((1-3)-ß-D-glucan [BG] detected in serum and CSF). Diagnosis of definite IE was retained according to Dukes revised criteria.[13,14] Infections were classified as disseminated when CNS involvement was associated with candidemia and/or another location of invasive candidiasis or localized when infection was only located in the CNS. We collected demographic and clinical treatment data and outcome from medical records in a standardized case report form in each center. Mortality was assessed crude (all death during the follow-up) and attributed to CNS involvement (death during ongoing infection with clinical or radiological progression). When available, serum and CSF samples were centralized for mannan antigen and anti-mannan antibody detection (Platelia *Candida* Ag. Plus/BioRad (limit of detection: index = 0.062; threshold for positivity: index = 0.125), Ac *Candida*: *Candida albicans* immunoglobulin G (IgG) ELISA/IBL (limit of detection = 12UA/ml; threshold for positivity = 25 UA/ml) and BG detection by means of the Fungitell® test kit (Associates of Cape Code, Inc. Falmouth, MA, USA) (positive cutoff value of 80 pg/ml). Tissue samples from brain biopsies were centralized at the Institut Pasteur, Histopathology Unit (Paris) for analysis and anti-*Candida* spp. staining. Immunohistochemistry (IHC) was carried out using a rabbit polyclonal primary antibody directed against *Candida albicans* (dilution 1:50000, GTX40096, Genetex, Taiwan). A centralized analysis of brain MRI was performed by a neuroradiologist.

Table 1. Underlying conditions and clinical characteristics of 24 patients with central nervous system candidiasis.

		Disseminated ($n = 17$)			
Patients and clinical characteristics	All ($n = 24$)	All ($n = 17$)	HM[a] ($n = 10$)	Non HM ($n = 7$)	Localized (n = 7)
Endocarditis (%)	10 (42)	10 (59)	3 (30)	7 (100)	0
Male sex (%)	14 (58)	11 (65)	5 (50)	6 (86)	3 (43)
Median age years (range)	51 [6–82]	52 [9–82]	51 [9–75]	52 [27–82]	50 [6–72]
Underlying conditions					
Neutropenia, month before (%)	9 (38)	9 (53)	9 (90)	0	0
Therapeutic LP[b]	7	7	7	0	0
Prosthetic valve (%)	4 (17)	4 (24)	0	4 (57)	0
IVDU[c] (%)	4 (17)	3 (18)	0	3 (43)	1 (14)
CARD9 deficiency (%)	2 (8)	0	0	0	2 (29)
Diabetes mellitus (%)	2 (8)	1 (14)	0	1 (14)	1 (14)
Recent neurosurgery (%)	2 (8)	0	0	0	2 (29)
HIV (AIDS stage)	1 (4)	1 (14)	1	0	0
Other (%)	2 (10)	1 (6)	0	1 (14)	1 (14)
Symptoms					
Fever (%)	20 (83)	14 (82)	10 (100)	4 (57)	6 (86)
Headache (%)	8 (33)	3 (18)	3 (30)	0	5 (71)
ICHT[d] signs (%)	7 (29)	1 (6)	1 (10)	0	6 (86)
Focal sign (%)	11 (46)	8 (47)	3 (30)	5 (71)	3 (43)
Impaired consciousness (%)	5 (21)	5 (29)	3 (30)	2 (28)	0
Ocular (%)	3 (13)	2 (12)	2 (20)	0	1 uveitis (14)
Positive blood cultures (%)	14 (59)	14 (82)	7 (70)	7 (100)	0
Extraneurological locations	12 (50)	12 (71)	9 (90)	3 (43)	0
> 1 location (%)	–	7 (41)	5 (50)	2 (28)	0
Spleen (%)	–	8 (47)	5 (50)	3 (43)	0
Liver (%)	–	4 (24)	4 (40)	0	0
Kidney (%)	–	5 (29)	4 (40)	1 (14)	0
Arthritis (%)	–	2 (12)	1 (10)	1 (14)	0
Laboratory meningitis (%)	8/13 (62)	2/7 (29)	2/7 (29)	0/0	6 (100)
MRI performed	n=19	n=12	n=7	n=5	n=7
MRI meningitis	7 (37)	2 (20)	1 (14)	1 (20)	5 (71)
Abscesses	11 (58)	7 (58)	5 (71)	2 (40)	4 (57)
Micro-abscesses[e]	9 (47)	7 (58)	5 (71)	2 (40)	2 (29)
Macro-abscesses	3 (16)	0	0	0	3 (43)
Empyema	1 (5)	0	0	0	1 (14)
Vascular complications	9 (47)	8 (67)	4 (57)	4 (80)	1 (14)
Haemorrhage	7 (37)	7 (58)	4 (57)	3 (60)	0
Ischemia	5 (26)	4 (33)	1 (14)	3 (60)	1 (14)
Aneurysm	0	0	0	0	0

For all median values, the range is given in square brackets.
[a]HM, hematological malignancy.
[b]LP, lumbar puncture.
[c]IVDU, intravenous drug user.
[d]ICHT, intracranial hypertension.
[e]Micro-abscesses measured less than 5 mm and macro-abscesses more than 5 mm.

The study was approved by our Institutional Review Board: Comité de Protection des Personnes (CPP) for the bio-collection, Commission Nationale de l'Informatique et des Libertés (CNIL) and Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (CCTIRS). All living subjects received written information and provided verbal informed consent.

Characteristics were analyzed as medians and range for continuous variables and as percentages for discrete variables.

Results

Twenty-four patients from 15 university hospitals (all with hematologic or transplant units) in France with CNS

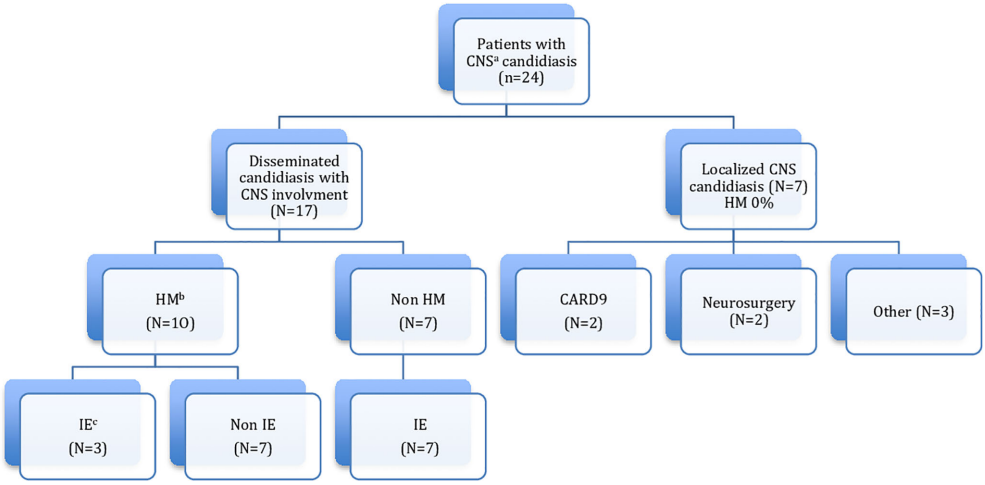

Figure 1. Flow chart of infection dissemination and risk factors. [a]CNS, central nervous system. [b]HM, hematological malignancies. [c]IE, infective endocarditis.

candidiasis were included; 23 cases were proven and one probable. The patient with probable infection was IVDU, had one CNS macro-abscess and uveitis highly suggestive of fungal origin, elevated ßDGlucan in CSF (>500 pg/ml) and favourable outcome with antifungal treatment.

Patients' characteristics

Their characteristics are reported in Table 1. Fourteen were male, with a median age of 51 years, including two children. Seventeen patients had disseminated candidiasis and seven localized infections (Fig. 1).

Disseminated infection

HM was the main risk factor present in 10 patients and included acute myeloid leukemia ($n = 2$), acute lymphoid leukemia ($n = 3$), lymphoma ($n = 4$), and aplastic anemia ($n = 1$). Nine out of the 10 patients with HM had prolonged neutropenia for a median of 21 days [12–30] before CNS candidiasis diagnosis. Seven patients had neutrophils below 500/mm^3 at time of diagnosis. One patient with Burkitt lymphoma was HIV-infected with HIV plasma viral load < 50 copies/ml at diagnosis and no other opportunistic infections. Among patients with HM, three had IE and seven did not. All seven patients without IE had been treated with at least one therapeutic lumbar puncture (LP) for HM, and three had concomitantly primary CNS lymphoma.

When not considering patients with HM, all seven other patients with disseminated candidiasis had IE. Underlying conditions were IVDU ($n = 3$, 43%), cancer (chemotherapy without neutropenia) ($n = 1$, 14%), or diabetes mellitus ($n = 1$, 14%). Four had prosthetic valve (two mechanical and two biological prostheses), one implantable cardioverter defibrillator, and one bicuspid aortic valve.

Localized infection

Underlying conditions were CARD9 deficiency ($n = 2$, 29%), diabetes mellitus ($n = 1$, 14%), IVDU ($n = 1$, 14%), and neurosurgery ($n = 2$, 29%). The two patients with CARD9 deficiency had history of chronic mucocutaneous candidiasis and were from consanguineous kindred. One Turkish patient had no identified risk factor and localized cerebral infection (meningitis with eosinophils and three macro-abscesses). CARD9 sequencing could not be performed as the patient died before description of this primary immunodeficiency. Healthcare-related infection after neurosurgery included one surgical site infection with extradural empyema and one ventriculoperitoneal (VP) shunt ventriculitis.

Clinical presentation

Clinical presentation is detailed in Table 1.

Disseminated infection

In case of disseminated infection, median time from first symptoms to CNS candidiasis diagnosis was 4 days [2–36 days], and two diagnoses were obtained at autopsy. Focal signs were frequent during IE (75%). Patients with HM had frequently multiple extra neurological locations as confirmed with autopsy results performed in two HM patients who had respectively 5 and 13 *Candida* spp. metastatic locations. One patient treated for lymphoma developed symptoms during neutrophil recovery with hepatosplenic and brain abscesses: diagnosis of chronic disseminated candidiasis was retained.

Localized infection

Median time from first symptoms to diagnosis was 23 days [3–89 days]. All evaluated patients had meningitis and clinical symptoms of ICHT, four confirmed by CSF pressure measurement, and one by papillary oedema.

Table 2. Laboratory characteristics of 24 patients with central nervous system candidiasis.

Laboratory characteristics	All ($n = 24$)	Disseminated ($n = 17$)		Localized ($n = 7$)
		HM[a] ($n = 10$)	Non HM ($n = 7$)	
CSF[b] analysis	$n = 13$	$n = 7$	$n = 0$	$n = 6$
Meningitis (%)	8 (62)	2 (29)	–	6 (100)
Median leucocyte count (/mm^3)	46 [3–410]	11 [3–20]	–	176 [9–410]
Median lymphocyte %	73 [29–96]	29	–	80 [65–96]
Median neutrophils %	27 [2–62]	62	–	20 [2–60]
Clinical ICHT[c]	7	1	–	6
IC[d] pressure (cmH2O)	–	–	–	35
Protein (g/L)	1.09 [0.19–5.69]	0.44 [0,19–1.09]	–	1.33 [0.36–5.69]
Hypoglycorachia (%)	5 (38)	2 (29)	–	3 (50)
Positive microscopic exam (%)	2 (15)	0	–	2 (33)
Positive culture (%)	4 (31)	1 (14)	–	3 (50)
Positive BG[e]	6/7	3/4	–	3/3
Median positive BG (pg/ml)	377 [122→500]	495 [122→500]	–	259 [151 – >500]
Positive *Candida* mannan Ag	4/5	2/2	–	2/3
Blood				
Median C reactive protein (mg/l)	108 [12–385]	135 [75–385]	98 [12–284]	2.7 [1–155]
Positive blood cultures (%)	14 (58)	7 (70)	7 (100)	0
Positive serum BG (%)	14/14 (100)	7/7 (100)	2/2 (100)	5/5 (100)
Median serum BG (pg/ml)	453 [95–500]	500 [95–500]	357 [214–500]	188 [145–407]
Positive mannan antigen and/or anti-mannan antibody (%)	10/14 (71)	4/6 (67)	2/2 (100)	4/6 (67)

For all median values, the range is given in square brackets.
[a]HM, hematological malignancy.
[b]CSF, cerebrospinal fluid.
[c]ICHT, intracranial hypertension.
[d]IC, intracranial.
[e]BG: (1-3)-ß-D-glucan.

Diagnosis

Patients' laboratory findings are detailed in Table 2. Cultures of normally sterile samples (blood, CSF, or CNS biopsy) grew mainly *C. albicans* (70%); other species were *C. tropicalis*, *C. parapsilosis* (two each) and *C. kefyr, C. glabrata, C. guilliermondii* (one each).

Disseminated infection

Among 17 patients with disseminated infection, 14 (82%) had positive blood culture. Candidemia preceded diagnosis of CNS infection for a median of 5.5 days, except for two patients with IE who had brain lesions detection 8.5 days before blood culture puncture. One diagnosis relied on liver biopsy, and two resulted from autopsies. Candidemia was prolonged for a median of 4 days during IE, range to [2–13] and 3 days for others, range to [1–7]. One patient had both blood and CSF positive cultures simultaneously. Two patients treated for HM had meningitis with low median CSF count of 11/mm^3 probably due to neutropenia at the time of procedure. BG was detected in serum of all patients and in CSF for three fourths of tested CSF with high median titer (453 and 377 ng/ml, respectively). The patient with negative BG in CSF had one *C. albicans* brain micro abscess during chronic disseminated candidiasis.

Localized infection

Proven diagnosis resulted from three positive CSF cultures and four cerebral biopsies. No patient had positive blood culture. By contrast with disseminated forms; all patients who had CSF analyses had high CSF cellularity (median CSF count of 176/mm^3) and ICHT. BG was positive in serum for all patients and for all tested CSF.

Imaging

MRIs were centrally reviewed for 19 patients: 12 with disseminated infections and seven with localized infections. Data are presented in Table 1.

All but two abscesses presented contrast enhancement as well as one ischemic lesion with hemorrhagic component (Fig. 2). The apparent diffusion coefficient (ADC) was low for 10 patients, corresponding to abscesses for eight cases and ischemic lesions during IE for two cases. Four patients had abscesses with high ADC: two had several abscesses with variable ADC (Fig. 3) and two had exclusively macro-abscesses with high ADC.

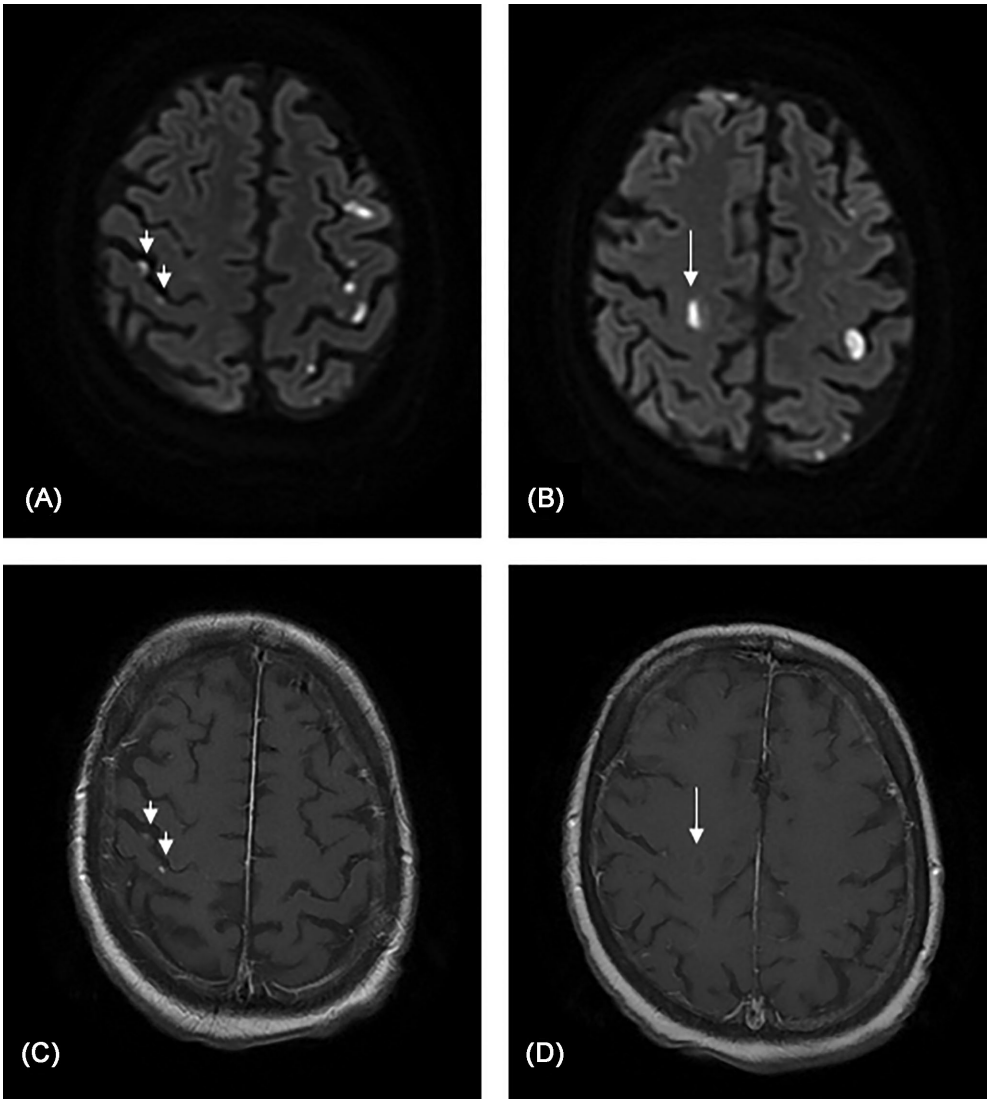

Figure 2. MRI of patient with *Candida* IE. Ischemic and hemorrhagic lesions secondary to multiple brain emboli progressing in micro-abscesses. Axial DWI images (b-values of 1000 s/mm^2) with several bilateral hypersignal evocating hematogenous diffusion (A, B). Gadolinium-enhanced axial T1-weighted image (C, D). One lesion with annular enhancement (white arrow) correspond to one abscess; two nodular enhancement (white arrowhead) correspond either to micro-abscesses or ischemic lesions with hemorrhagic component. Other hypersignals with no enhancement are punctiform ischemic lesions.

Disseminated infection

Patients with disseminated infections had: MRI meningitis (2/12, 20%), micro-abscesses (7/12, 58%) (supratentorial [$n = 7$, 58%], subtentorial [$n = 2$, 20%]), or vascular complications (8/12, 67%). No patient had macro-abscess. Patients with IE had ischemia (4/6, 67%) and or hemorrhage (4/6, 67%) (Fig. 2). By contrast, patients without IE had micro-abscesses (5/6, 83%), frequently more than 10. Only two patients had vascular complications due to abscesses hemorrhages (Fig. 3).

Localized infection

For patients with localized CNS infections, MRI showed micro- and/or macroabscesses ($n = 4$, 57%) (supratentorial [$n = 3$], or in spinal cord [$n = 1$]), meningitis ($n = 5$, 71%) and frequently both ($n = 3$, 43%). Two patients had isolated meningitis and ventriculitis without abscesses: one spontaneously, the other secondary to VP shunt. The two CARD9 deficient patients had more than 10 localized micro-abscesses (Fig. 4). For one, they converged in a macro-abscess. One patient with chronic meningitis had an ischemic cerebrovascular accident; no vasculitis was evidenced on angiography.

Histopathology

Four brain samples were analyzed (Fig. 5). Patients with a CARD9 deficiency ($n = 2$) displayed heterogeneous lesions: (i) (A) small, randomly distributed, infiltrates of macrophages, lymphocytes and plasma cells with perivascular cuffing, (ii) (D) focal granuloma with giant multinucleated cells. Hyaline fungi were detected in the lesions, with the association of yeasts,

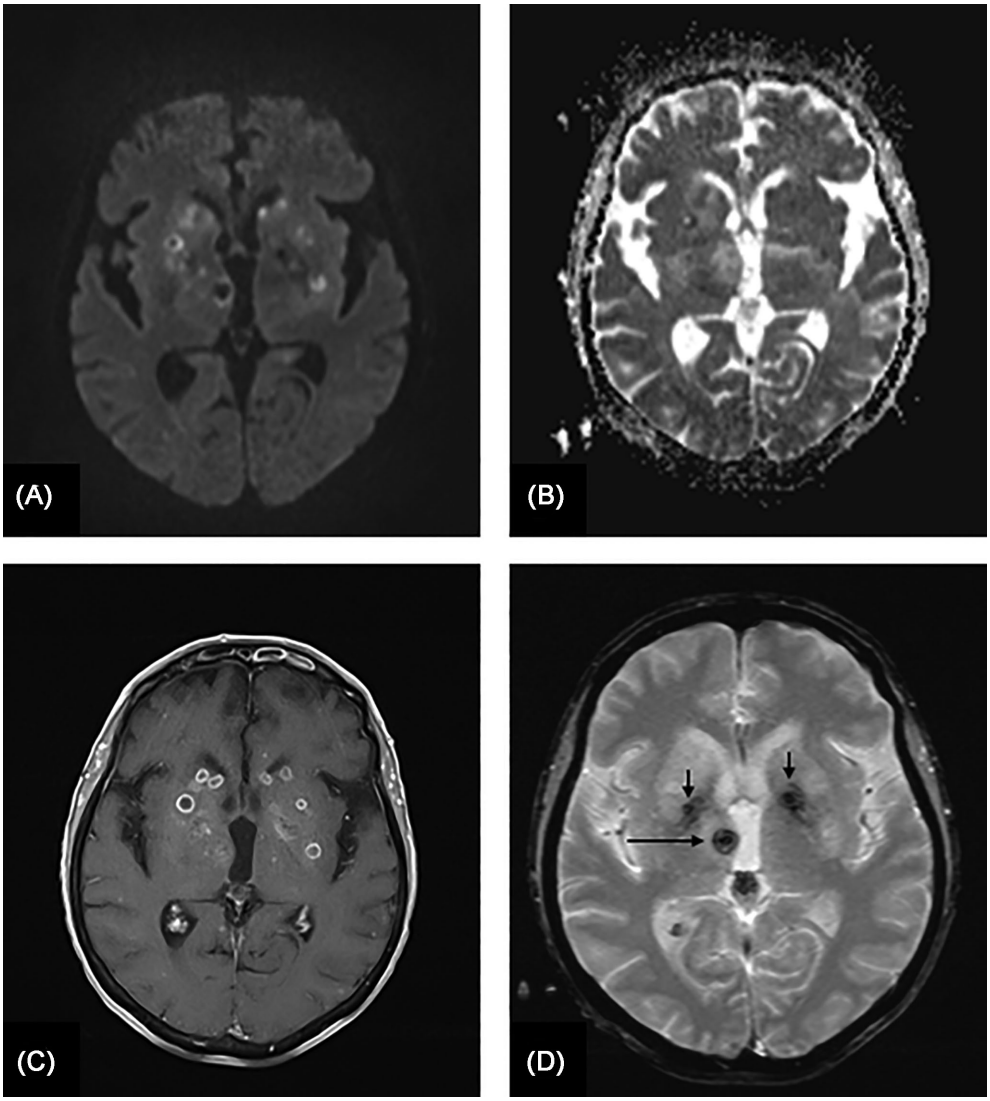

Figure 3. MRI of a patient with disseminated infection without IE during neutropenia. (A) Diffusion Weighted Imaging (DWI) (with b-values of 1000 s/mm^2) (A) and (B) ADC cartography show multiple micro and macro abscesses in the corpus callosum with a variable diffusion signal and cartography ADC value. (C) All micro-abscesses enhance on gadolinium-enhanced 3DT1-weighted image. (D) Axial T2* image shows micro-abscess hemorrhagic phenomena (black arrow) and basal ganglia common calcifications (black arrowhead).

pseudo-hyphae and thin, septate hyphae with rare branching (only small fragmented hyphae for one patient). The patient without predisposing (suspicion of CARD9 deficiency) (C) had large necrotic areas with blood vessel destruction or thrombosis, and infiltration of neutrophils and macrophages at the periphery; anti-*Candida* immunohistochemistry was positive. By contrast, (B) the patient with HM displayed large cerebral infarction, with fungus invasion of the necrotic tissue and blood vessels (thrombo-embolic origin). (F, G) Yeasts, pseudo-hyphae, and thin, septate hyphae with rare branching were identified. Anti-*Candida* immunohistochemistry (H) was also positive.

Treatment and outcome

All patients received antifungal treatment (Table 3). Initial treatment after diagnosis of CNS infection was a combination for 14 patients (with liposomal amphotericin B [L-AmB] and flucytosine [5FC] for most patients [$n = 11$] L-AmB and voriconazole [$n = 1$] or caspofungin and 5FC [$n = 2$]) and monotherapy for 10 with azole ($n = 4$), L-AmB ($n = 3$), or echinocandin ($n = 3$). During follow-up, 19 patients received L-AmB in association with 5FC for a median of 19 days, range to [4–92 days]. Two patients received exclusive monotherapy with fluconazole 800 mg daily. Median total treatment duration was 82 days, range to [3–393 days].

Five out of seven patients with localized CNS infections had a neurosurgical procedure: two abscesses drainages and four extraventricular drain (EVD). Overall, five patients with localized infections had ICHT treatment: one with repeated LP and four with EVD. One patient death was related to intracranial hypertension.

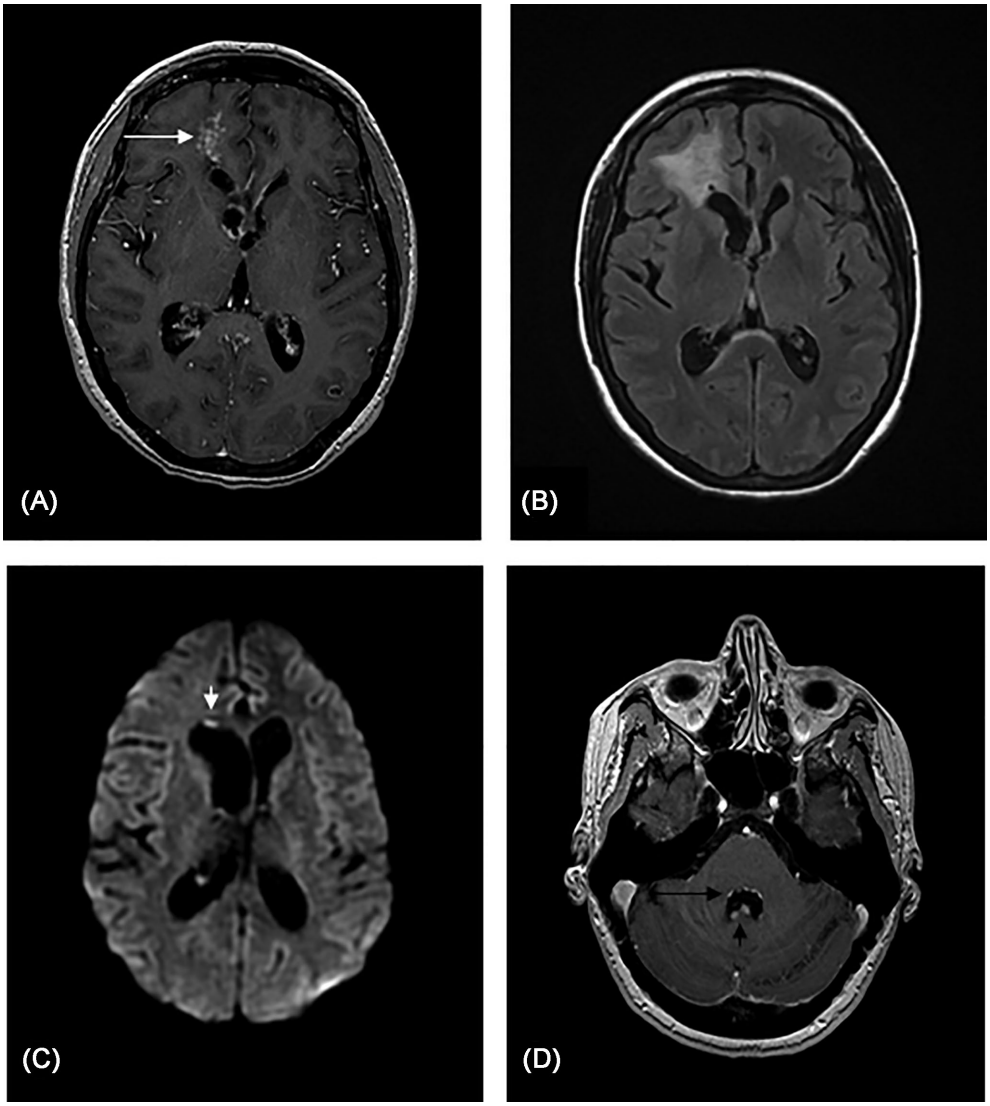

Figure 4. MRI of a patient with CARD9 deficiency and localized CNS infection. Gadolinium-enhanced 3DT1-weighted image (A) show multiple right frontal and periventricular white matter micro-abscesses with enhancement (white arrow) and perifocal edema on axial T2 flair image (2). One micro-abscess with high signal on DWI (b-values of 1000 s/mm^2) (white arrowhead) (C). Gadolinium-enhanced 3DT1-weighted image (D) shows an aspect of nodular meningitis (black arrow) with micro abscesses (black arrowhead).

Concerning patients with IE, three underwent valve replacement. None of these patients had HM and all had favorable outcome. No other patient with *Candida* IE had valve replacement due to risk of complications (neutropenia, brain hematoma for three patients) or absence of significative valvular lesions (diagnostic retained with Dukes revised criteria). Among them, four of seven died with progressive infection. No patient relapsed after 10 months of follow-up.

Overall, 14 patients (58%) died during follow-up, with 10 deaths (42%) related to *Candida* infection (death due to septic shock or neurological deterioration). *Candida*-related deaths were more frequent in case of HM (70%). Forty percent of patients with IE died. One patient with localized CNS infection died due to relapse of brain abscesses 4 months after starting treatment.

Discussion

We identified 24 cases of CNS candidiasis in France during a 13-year period. The study is not exhaustive but highlights the rarity, severity, and challenges to diagnose this infection. Previous studies were case reports (single or few cases) and review of literature including several autopsy cases.[6,15–17] Our original results allowed us to better decipher CNS candidiasis characteristics according to underlying host characteristics with the evidence of three distinct entities: (i) CNS microabscesses as a consequence of hematogenous dissemination in HM patients receiving therapeutic LP complicated with subsequent high mortality rate; (ii) CNS vascular lesions in case of *Candida* IE; and (iii) localized CNS candidiasis with macro-abscesses and meningitis complicated with ICHT, with presence of *Candida* yeasts and elevated

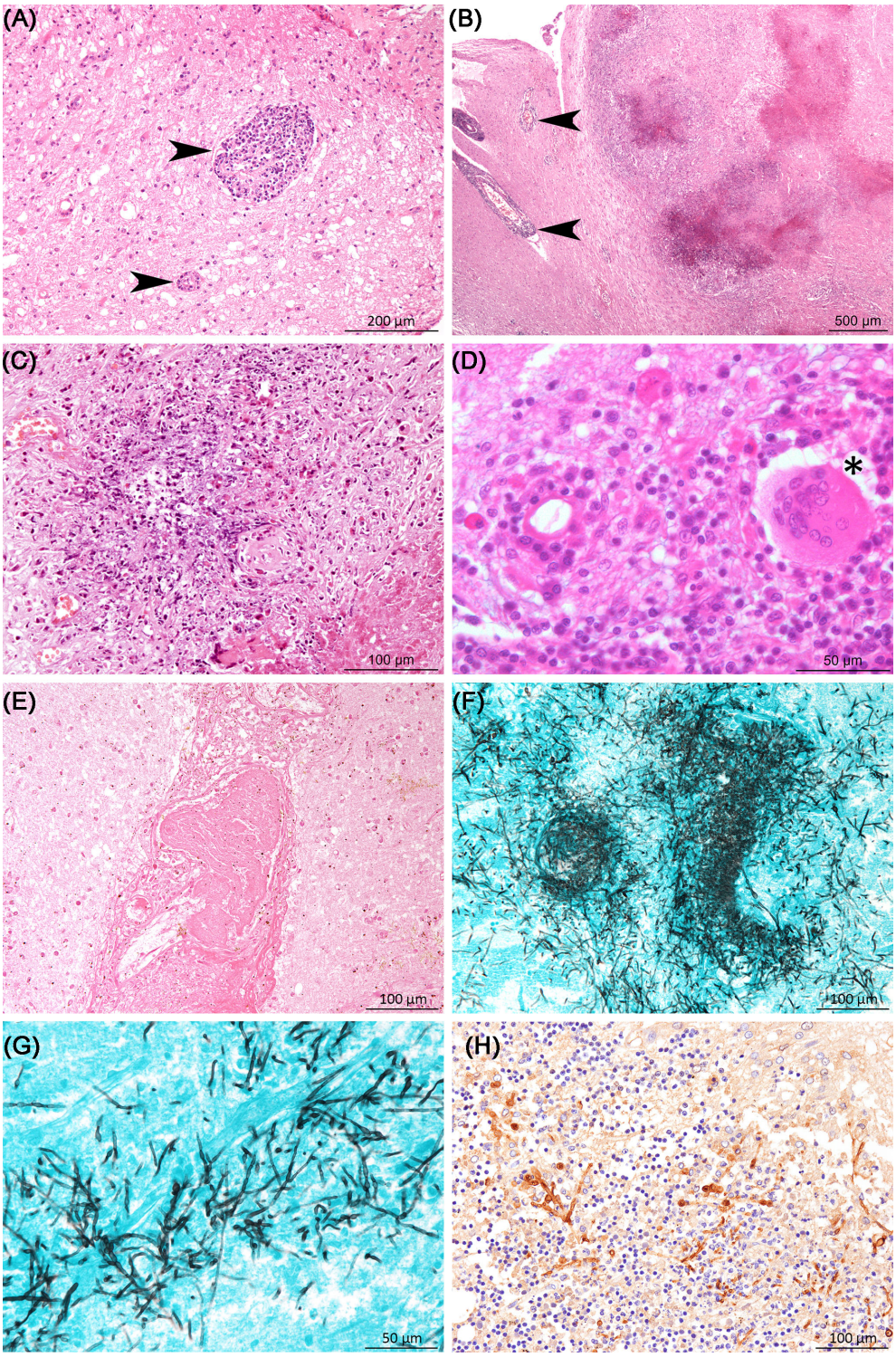

Figure 5. Histopathological analysis. Four brain samples were analyzed; we observed lesions with varying severity, from mild encephalitis to massive destruction of the brain tissue. (A) Mild lesions were characterized by multifocal, randomly distributed, perivascular cuffs of lymphocytes and plasma cells (arrowheads), with diffuse extracellular edema. (B) Severe lesions associated perivascular cuffs (arrowheads) and large areas of tissue destruction: (C) presence of necrosis, gliosis, and diffuse infiltration of lymphocytes, plasma cells and macrophages. (D) Granulomas could also be detected with giant multinucleated cells (star). For two samples, severe vascular lesions were observed, characterized by (E) complete thrombosis sometimes associated with necrosis of the vessel wall, leading to ischemic necrosis of the brain tissue (infarct). Hyaline fungi (yeasts and hyphae) were identified in the 4 samples, using Gomori Grocott staining, either (F) filling necrotic blood vessel spaces, or (G) invading the brain tissue. (H) Anti-Candida immunohistochemistry analysis was positive for two samples. A, B, C, D, E: HE staining. F, G: Gomori Grocott staining; H: Anti-*Candida* immunohistochemistry. A, D: patients with CARD9 mutation.

Table 3. Treatment and outcome of 24 patients with CNS candidiasis.

Treatment and outcome	All ($n = 24$)	Disseminated ($n = 17$)		Localized ($n = 7$)
		HM[a] ($n = 10$)	Non HM ($n = 7$)	
Antifungal treatment				
LAmB[b] (%)	19 (79)	9 (90)	5 (71)	5 (71)
Median days LAmB	23 [11–92]	28 [11–47]	14 [4–52]	26 [14–92]
5FC (%)	19 (79)	8 (80)	6 (86)	5 (71)
Median days 5FC	19 [4–92]	24 [6–44]	14 [4–65]	14 [6–92]
Fluconazole (%)	17 (71)	6 (60)	6 (86)	5 (71)
Echinocandin (%)	6 (25)	3 (30)	3 (43)	0
Combination (%)	20 (83)	9 (90)	6 (86)	5 (71)
Median days AF duration	82 [3–393]	36 [3–158]	103 [26–365]	180 [57–393]
Secondary AF prophylaxis (%)	4 (17)	1 (10)	1 (14)	2 (29)
Surgery				
EVD[c] (%)	4 (17)	0	0	4 (57)
Neurosurgery (%)	2 (8)	0	0	2 (29)
Evolution				
Sequela (%)	3 (30)	0	0	3 (50)
Death at 3 months	7 (29)	6 (60)	1 (14)	0
Death with evolutive infection (%)	10 (42)	7 (70)	2 (29)	1 (14)

For all median values, the range is given in square brackets.
[a]HM, hematological malignancy.
[b]LAmB, liposomal amphotericin B.
[c]EVD, extra ventricular derivation.

Candida biomarkers in CSF, associated with low mortality rate and frequent sequelae.

All patients with HM and hematogenous dissemination not related to IE, received therapeutic LP before infection, among which three had lymphoma localization. We therefore assume that blood-brain barrier was damaged by LP and intrathecal chemotherapy or CNS lesion and associated with local immunodepression; facilitating *Candida* spp. dissemination to CNS during candidemia in this patient population. We then underlie the importance to perform brain MRI in patients with candidemia who recently had therapeutic LP. This risk factor could explain the important number of patients with HM in our study compared to French epidemiology of patients with candidemia. Analysis of candidemia in a French active hospital-based surveillance program (Paris, 2002 to 2014) revealed 3417 candidemia: only 17.1% had HM.[18]

During *Candida* spp. IE, CNS embolic complications are described in 12 to 22% of patients.[7,10,11] In one old autopsy series, 48% of patients with disseminated candidiasis had cerebral infections.[4] Brain MRI is therefore also necessary for patients with IE because CNS involvement is probably underdiagnosed.

Among seven patients with localized CNS candidiasis, all presented subacute meningitis. One patient had diabetes mellitus, and two had CARD9 deficiency. CARD9 deficiency was identified in two cases and could not be explored in a third because patient deceased. Autosomal recessive CARD9 deficiency is a primary immunodeficiency electively predisposing to fungal infections including CNS candidiasis with 17 cases reported to date.[9,19–25] CARD9 deficiency is responsible for specific defect of immunity in the CNS with abnormal chemotactism of polymorphonuclear leukocytes due to specific microglia immune defect in response to *Candida* spp.[5,26] Our series confirms that CARD9 deficiency should be explored in cases of CNS candidiasis without known predisposing conditions, whatever the age. Previous studies described 28% of patients with no underlying illness predisposing to candidiasis during chronic *Candida* spp. meningitis. Many of these patients could have unknown primary immunodeficiency including CARD9 deficiency or chronic granulomatous disease.[17] History of chronic mucocutaneous candidiasis and consanguinity are suggestive of this primary immunodeficiency. Two cases occurred after neurosurgery in our series, probably resulting from contamination during the procedure, has previously described in the literature.[8,27]

In our study, half of the patients had cerebral abscesses, frequently multiple in concordance with the literature where *Candida* abscesses are more likely small and multiple than bacterial abscesses.[6,28] On MRI, most patients had abscesses with low ADC, as classically described for pyogenic and fungal cerebral abscesses.[29,30] They corresponded to lesions with true restricted diffusion on diffusion weighted imaging (DWI). By contrast, four patients presented abscesses with variable or high ADC values. Similar results are described by Mueller-Mang and colleagues. They retrospectively compared nine patients with fungal brain infections and 17 patients with pyogenic brain abscesses and found that all fungal abscesses showed higher ADC

values than bacterial abscesses.[31] In a recent study describing 13 cases of cerebral aspergillosis secondary to hematogenous dissemination, lesions were abscesses with presence of target-like ADC signal with central high ADC values, accounting for approximately 5% of the lesions.[32] DWI appears to be the most sensitive modality for identification of cerebral fungal abscesses.

CSF analysis was helpful for CNS candidiasis diagnosis, mostly for localized forms. All patients with localized infections had laboratory evidence of meningitis with frequently positive microscopic exam and/or culture, by contrast with two in disseminated forms, which is concordant with previous studies.[15] CSF cellularity was high with lymphocytic predominance. In previous studies, CSF cellularity and cell types varied widely with either neutrophile or lymphocyte predominance.[16,27,33] Proteins were also usually discreetly high, as previously described.[15,16] Half of the patients had positive CSF direct exam and/or culture on the second LP, underlying the importance to repeat exams or to draw sufficient volume. Furthermore, mannan or BG were frequently detected on CSF. In localized infections BG levels were higher in CSF than blood, and serum levels were lower than in disseminated infections supporting absence of hematogenous candidiasis in those forms. CSF BG benefit was previously reported during the outbreak of fungal meningitis associated with contaminated methylprednisolone with a sensitivity and specificity of 96% and 95%, respectively, for proven meningitis using the manufacturer's cutoff value (80 pg/ml).[34,35] CSF *Candida* mannan Ag was positive in four of five patients in our study as in one report where mannan was detected in the CSF of four patients with proven CNS candidiasis,[36] suggesting that this test can help for CNS candidiasis diagnosis. Consequently, CSF analysis including repeated LP with large volume with direct exam, culture, BG, and mannan could improve CNS candidiasis diagnosis.

Most patients received treatment with a combination of L-AmB and 5FC and step-down therapy with fluconazole that is consistent with IDSA guidelines.[37] The rationale for the initial combination is to achieve a synergistic effect against *Candida* and the association of L-AmB and 5FC should be maintained at least 14 days with repeat LP to confirm improvement and prolonged if CSF culture remained positive. Amphotericin B is used because of its fungicidal activity against *Candida* species, even though drug levels in the CSF and brain are low; flucytosine is added because of its anti-candidal activity and excellent penetration into CSF and brain tissue.[38,39] Fluconazole and voriconazole are proposed as step-down therapy because of good brain penetration and availability of oral formulation.[40] Caspofungin has relatively poor CSF penetration and therefore is not recommended.[41] Patients with CARD9 deficiency should have suppressive long-term treatment due to risk of relapse. In case of IE, long-term suppression is also proposed for patients who cannot undergo valve replacement.[14,37] In our study, three patients achieved IE treatment without valve replacement. Only one had suppressive long-term treatment and none relapsed. ICHT was an important concern for localized infections. It's therefore necessary to monitor CSF opening pressure (OP) during LP and perform repeated depleting LP to decrease OP. In our study, three patients had repeated LP; they were insufficient for two who required EVD. One patient with non-healthcare-associated infection had surgical resection of the macro-abscess, but he relapsed 7 months later. Mortality attributed to CNS involvement is high (42%), particularly in HM patients without IE.

Limitations of the study was the retrospective design with various methods of data retrieval by local investigators; prolonged period of cases inclusions with evolution in diagnostic methods (such as BG); knowledge about immunodeficiency predisposing (such as CARD9) and treatments. Our data collection relies on voluntary declarations is not exhaustive. Data from prospective surveillance would be necessary to evaluate incidence.

Our original results allowed to better decipher CNS candidiasis characteristics with distinct entities related to host factors.

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