Cerebral aspergillosis in the era of new antifungals: the CEREALS national cohort study Nationwide CEREbral Aspergillosis Lesional study (CEREALS)

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Nationwide CEREbral Aspergillosis Lesional study (CEREALS)

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Key words : cerebral aspergillosis, galactomannan, invasive fungal disease

Abstract

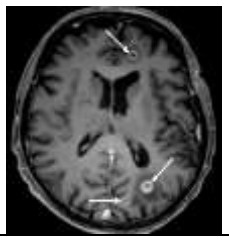
Background: Cerebral aspergillosis (CA) is a life-threatening disease for which diagnosis and management remain challenging. Detailed analyses from large cohorts are lacking.

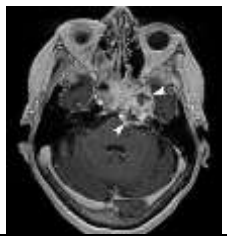
Methods: We included 119 cases of proven (n=55) or probable (n=64) CA diagnosed between 2006 and 2018 at 20 French hospitals. Data were collected at baseline and during follow-up. Cerebral imaging was reviewed centrally by two neuroradiologists.

Results: The most frequent underlying conditions were haematological malignancy (40%) and solid organ transplantation (29%). Galactomannan was detected in the serum of 64% of patients. In 75% of cases, at least one of galactomannan, *Aspergillus* PCR, and β-D-glucan was positive in the cerebrospinal fluid. Six-week mortality was 45%. Two distinct patterns of disease were identified according to presumed route of dissemination. Presumed haematogenous dissemination (n=88) was associated with a higher frequency of impaired consciousness (64%), shorter time to diagnosis, the presence of multiple abscesses (70%), microangiopathy (52%), detection of serum galactomannan (69%) and *Aspergillus* PCR (68%), and higher six-week mortality (54%). By contrast, contiguous dissemination from the paranasal sinuses (n=31) was associated with a higher frequency of cranial nerve palsy (65%), evidence of meningitis on cerebral imaging (83%), macrovascular lesions (61%), delayed diagnosis, and lower six-week mortality (30%). In multivariate analysis and in a risk prediction model, haematogenous dissemination, haematological malignancy and the detection of serum galactomannan were associated with higher six-week mortality.

Conclusion: Distinguishing between hematogenous and contiguous dissemination patterns appears to be critical in the workup for CA, as they are associated with significant differences in clinical presentation and outcome.

Graphical abstract





	Haematogenous dissemination	Contiguous infection
Population characteristics	median age: 56 y [44-62 y] Peculiar population : HM or SOT (77%)	median age: 68 y [55.5-77 y] Peculiar population : diabetes or no immunosuppression (39%)
Median time between onset of symptoms and diagnosis	2 days [0-9]	54 days [16-126]
Mycology	Positive assays for GM (69%) and *Aspergillus* PCR (68%) in serum	Negative assays GM (54%) and *Aspergillus* PCR (100%) in serum
Radiological findings	Abscesses (95%), often multiple (69%), with microangiopathy (52%)	Radiological meningitis (83%) and macrovascular lesions (61%)
Mortality	Higher (54% at W6, 69% at M12)	Lower (19% at W6, 46% at M12)

Summary of clinical, mycological and radiological major characteristics of CA

Highlights :

- Characteristics of cerebral aspergillosis vary according to the presumed route of *Aspergillus* dissemination to the brain
- Galactommanan antigen is positive in the sera in 61% of the patients and in the CSF in 69%
- CSF analysis is often informative and should be formed systematically if possible
- Cerebral ischemic events are reported in 2/3 of patients with cerebral aspergillosis with contiguous extension
- Cerebral aspergillosis mortality remains high (45% at W6 and 63% at M12)

Introduction

Cerebral aspergillosis (CA) is one of the most severe forms of invasive aspergillosis (IA), and can occur after presumed haematogenous dissemination from an extra-cerebral site, by contiguous extension from an infection of the paranasal sinuses or the ears, or by direct inoculation (1). The majority of cases of CA reported in the literature concerns highly immunocompromised patients, such as neutropenic patients with haematological malignancy (HM) or solid organ transplant recipients (SOT) (2,3). There have also been reports of CA in moderately immunocompromised patients such as those with diabetes mellitus, and even in patients with no known risk factors (4,5). The clinical, radiological, and mycological features associated with the different underlying diseases or the route of dissemination to the brain (contiguous vs hematogenous) are poorly characterised. A few small series of cases have correlated brain Magnetic Resonance Imaging (MRI) patterns with the underlying immune status of CA patients (6,7). Data are also lacking on the efficacy of therapeutic strategies. Voriconazole and isavuconazole form the cornerstone of treatment, but the place of first-line dual therapy or of surgery and its timing in the treatment of CA remains a matter of debate (4,8–10).

We therefore conducted a nationwide study with the primary objective of assessing the impact of the presumed route of dissemination to the brain on the clinical and radiological presentation, as well as the six-week mortality in CA.

Methods

Type of study and data sources

We performed a retrospective study in France between January 2006 and December 2018: the CEREbral Aspergillosis Lesional study (CEREALS). Patients were identified through

mycologists, haematologists and specialists in infectious diseases or intensive care. Cases were identified at each participating centre, through physician notifications and cross-checking data with local microbiological databases. Clinical and radiological data were recorded on a standardised case report form. All brain computed tomography (CT) and/or MRI scans performed within 30 days before or after the diagnosis of CA and available were centrally reviewed by two neuroradiologists (JB and ON) blind to patient outcome.

Patients and definitions

We included patients with proven or probable CA, as defined by the 2019 updated EORTC/MSG criteria, with the addition of diabetes mellitus as a host criterion (11). Proven CA was defined either as positive culture from the cerebrospinal fluid (CSF), a positive culture or histopathology from brain tissue with a positive tissue PCR results or, for patients with a sinus infection, histological evidence of invasive mold infection associated with growth of *Aspergillus* in culture from a sinus biopsy and imaging showing a continuity of the infection from the sinus to the brain. Probable CA was defined as focal brain lesions or meningeal enhancement on imaging associated either with the detection of GM in the CSF, or with proven or probable invasive extracerebral aspergillosis. As diabetes mellitus does not belong to EORTC/MSG host criteria, the qualification “modified probable” CA was retained in cases of diabetes being the only host criterion.

The presumed route of *Aspergillus* dissemination to the brain was classified as haematogenous or contiguous based on radiological data. We defined contiguous infections as those occurring as a result of the extension to the brain of a previously paranasal or ear infection, or occurring following facial trauma with radiological evidence of bone lysis. All other cases were considered as consequences of haematogenous dissemination.

Underlying diseases: Eight main underlying diseases were considered, with a single disease assigned to each patient, in the following order: haematological malignancy (HM) (including haematopoietic allogeneic stem cell transplantation), solid organ transplantation (SOT), diabetes mellitus, auto-immune or systemic inflammatory disease requiring corticosteroids or immunosuppressive treatment, a history of neurological or sinus surgery in the last three months, HIV infection, cirrhosis and primary immunodeficiency.

Biological definitions: Galactomannan antigen (GM) was considered "at diagnosis" when measured with 15 days prior or after CA diagnosis. It was considered positive above 0.5 in the sera and in the CSF. However, for the diagnosis of probable IA, a threshold of 1 was used, in accordance with the 2019 update of the EORTC/MSG criteria (11). β-D-glucan was considered positive when above 80 pg/mL in the sera or the CSF. Neutropenia was defined as a neutrophil count below 500/mm^3 and lymphopenia was defined as a lymphocyte count below 1000/mm^3. Meningitis was defined as a CSF white blood cell count ≥ 5/mm^3.

Imaging: The following imaging features were evaluated: type of dissemination (haematogenous vs. contiguous) according to lesion location, number, location and peripheral enhancement of abscesses, presence of ventriculitis, brain haematoma, ischemic lesions, lepto- and/or pachymeningitis (when post-contrast imaging was performed), presence and number of micro-bleeds (when T2* or susceptibility-weighted images were performed) (see in supplementary methods for details definitions). Arterial stenosis or occlusion, arterial aneurysm were searched for on CT-Angiography or Time of Flight MR, when available. On imaging, abscesses were defined as limited intra- or extra-axial lesions with or without peripheral enhancement (wall enhancement). Brain infarct was defined as focal high intensity area without contrast enhancement on Diffusion-Weighted Imaging when available and/or lesions involving arterial territory hypodense on CT or hyperintense

on Fluid-attenuated Inversion Recovery (FLAIR) MRI. Small vessel disease was defined as the presence of ≥ 5 microbleeds in the absence of thrombopenia (<50 G/L) or a history of high blood pressure and/or lacunar infarct and/or deep nucleus haematoma (17). Large vessel disease was defined as the presence of arterial stenosis and/or occlusion and/or large brain infarction involving arterial territory.

Treatment definitions: The first day of treatment was defined as the first day on which the patient received a drug active against *Aspergillus* spp. for the treatment of cerebral aspergillosis. Dual therapy was defined as treatment, for at least seven days, with two antifungal drugs active against *Aspergillus* spp.

Statistical analysis

The primary outcome was 6-week mortality, with follow-up counted from the date of diagnosis of CA. It was treated as a binary outcome, with censoring before 6 weeks (1 patient) handled by weighting by the inverse probability of censoring (12). Missing baseline data were handled by multiple imputations based on chained equations, with 10 imputed datasets. Logistic regression was used to estimate the association of a set of predefined variables (age at diagnosis, sex, underlying disease, neutropenia at diagnosis, time from first symptoms to diagnosis, and detection of GM in serum) with 6-week mortality. The development and internal validation of the model are described in the supplementary material. Due to the limited number of events, we used different strategies for model building, based on backward variable selection. The final model is presented with odds ratios obtained after shrinkage and their 95% confidence intervals (95% CI). Given the exploratory objective of comparison between patients with hematogenous vs. contiguous extension, no correction for multiple testing was used. All tests were two-tailed, and *p* values of 0.05 or

less considered statistically significant. Analyses were performed with R statistical software version 3.6.3.

Ethical considerations

The study was approved by the institutional review board of Necker-Enfants Malades Hospital (2017-AS-13) and was declared to the "Commission Nationale de l'Informatique et des Libertés" in accordance with French law. All clinical data were recorded anonymously.

Data Availability Statement

Anonymized data will be shared by request from any qualified investigator.

RESULTS

Patient characteristics

In total, 119 patients with CA (54 proven, 65 probable) from 20 French hospitals were included in this study (figure 1). Seven patients receiving ibrutinib were reported in a previously published study (13). The main baseline characteristics of patients are presented in table 1.

Sixty-six per cent were male and median age at diagnosis was 58 years [47.5-66.5]. Seven patients were children. The most common underlying diseases were HM (n=47; 40%), followed by SOT (n=34; 29%), diabetes (n=10; 8%) and auto-immune diseases (n=8; 7%).

CA occurred by presumed haematogenous dissemination in 88 patients (74%) and by direct extension from a contiguous site in 31 patients (26%). Patients with haematogenous dissemination were younger (p<0.001) and were more likely to have HM or SOT as a risk

factor (p<0.001) than those with contiguous infection, which was more frequent in patients with diabetes mellitus (p=0.02) or with no identified risk factor (p=0.009).

Clinical presentation

The clinical findings at diagnosis are presented in table 1. Sixty-four patients (54%) were hospitalised in an intensive care unit at the time of CA diagnosis.

Fever was present in 42% of the patients. The main neurological symptoms reported were impaired consciousness (46%), headache (35%), cranial nerve palsy (31%), seizure (21%), a focal sign such as motor deficit of the limb, frontal syndrome, cerebellar syndrome or aphasia (27%) and signs of cerebral hypertension (3%). The median time between onset of neurological symptoms and diagnosis of CA was 4 days [0-18]. Fourteen patients (11.7%) had no neurological sign at diagnosis.

Clinical presentation varied mainly according to *Aspergillus*' route of dissemination to the brain

Impaired consciousness was more frequent in cases with hematogenous dissemination (53% vs 36%, p=0.006), whereas cranial nerve palsy and headache were more frequent in cases of contiguous infection (65% vs 19%, p<0.001 and 65% vs 24%, p=0.01, respectively).

Neutropenia was present at diagnosis in 18 patients (15%). Comparison between neutropenic and non-neutropenic patients showed no differences, except for fever which was more frequently observed in the neutropenic group (13/18 [72%] vs. 35/101 [35%], p=0.004) (table S1).

Comparison of clinical findings according to underlying diseases (HM/SOT/other) showed that fever was rare in SOT patients (5/34 [15%] vs. 26/47 [55%] in the HM group, p=0.0002

and 17/38 [45%] in the remaining population, p=0.009) (table S2). Conversely, impaired consciousness was more frequently observed in SOT that in HM patients (22/34 [65%] vs 16/47 [34%], p=0.006).

Fungal culture, biomarkers and cerebrospinal fluid characteristics

Considering all the samples analysed (including extracerebral samples such as broncho-alveolar fluid), *Aspergillus* species were identified in 86 patients (table 2). *A. fumigatus* was the most frequent species (88% of all isolates). All *Aspergillus flavus* isolates were found in the contiguous group (p=0.004). A concomitant bacterial or fungal infection was diagnosed in 15 and 13 patients, respectively; a detailed description is provided in the supplementary material (table S4).

Sixty-seven patients (56%) had a lumbar puncture at diagnosis. All of the 67 CSF mycological cultures performed were negative, although CSF analysis showed evidence of meningitis in 25/58 cases (43%) (table 1). In cases of meningitis, median CSF WBC count was 258/mm^3 [70-885] with a predominance of polynuclear neutrophils in 76% of samples.

In the sera, 66/104 (64%) of the patients had a positive GM at diagnosis, 15/18 (83%) had a positive ß-D-glucan, and 15/27 (56%) a positive *Aspergillus* PCR (Table 2). Median titer of positive GM and of ß-D-glucan were 2.25 [1.05-4.7] and 431 ng/ml [188->500], respectively.

In CSF, 31/45 (69%) of patients had a positive GM, 7/11 (64%) a positive ß-D-glucan and 14/26 (54%) a positive *Aspergillus* PCR. Median index of the positive GM and ß-D-glucan in the CSF were 5 [1.1-7.8] and 359 [194->500], respectively (Table 2). At least one of the three biomarkers of CA was detected in the CSF for 40/53 patients (76%). In 6/18 cases (33%), the GM and *Aspergillus* PCR results were discordant in the CSF. No correlation was found between the presence of meningitis and the detection of GM in the CSF (*p*=0.73).

Serum but not CSF biomarkers positivity varied according to Aspergillus' route of dissemination

GM was more frequently detected in the serum of patients in the haematogenous dissemination group than in the contiguous group (55/80 [69%] vs 11/24 [46%], *p*=0.04), as well as in neutropenic patients compared to non-neutropenic patients (15/18 [83%] vs 51/86 [59%] *p*=0.06) (table 2, S1 and S3). In contrast, GM positivity in the serum did not vary significantly according to the underlying disease (Table S3). Positive assays for *Aspergillus* PCR in the serum were found exclusively in patients from the haematogenous dissemination group (n=15/22 [68%] vs 0/5 [0%], *p*=0.01). No differences according to the route of dissemination was found for ß-D-glucan.

GM, *Aspergillus* PCR and ß-D-glucan positivity in the CSF were similar in the haematogenous dissemination group and in the contiguous infection group (table 2).

Brain imaging characteristics

We were able to recover and analyze diagnostic cerebral imaging for 86 patients: 68 MRI and 18 CT-scan. Good quality imaging exploring the cerebral vessels was available for 53 patients. Radiological findings are displayed in Table 3. Brain abscesses were the most frequent lesion of CA, seen in 76% of the patients. Meningitis was present in 38% of patients. Six cases of ventriculitis were reported (7%), mostly in highly immunocompromised patients (5/6 had HM or SOT). In addition to brain lesions, one patient had a concomitant spinal infection (and 2 others developed a spinal location during the follow-up). Median number of abscesses per patient was 2 [1-4]. Nineteen patients (31% of the patients with abscesses) had a single lesion and 30 (48%) had more than 3. Median size of the main

abscess was 21 mm [15-29]. Abscess wall enhancement was seen in 84% of cases, which did not differ between the haematogenous dissemination and contiguous infection groups, between neutropenic and non-neutropenic patients, or according to the underlying disease (table 3, S2 and S3).

Two patterns of radiological lesions were associated with Aspergillus' route of dissemination to the brain

Brain abscesses (p<0.001), particularly multiple (>3) (p=0.02), were more often observed in the haematogenous dissemination group than in the contiguous infection group (Table 3). On the contrary, a contiguous infection was associated with a higher frequency of pachymeningitis (p<0.001) (figure 2). Large vessel disease was more frequently detected in cases of contiguous infection (p=0.01), mainly as the consequence of skull base and cavernous sinus involvement, whereas small vessel disease was more frequently associated with haematogenous dissemination (p=0.002) (figure 2). Occlusion of a large venous sinus was only seen in case of contiguous dissemination, as a consequence of direct vein invasion.

Treatment

In total, 113 patients received antifungal agents. Details regarding the first-line treatments are displayed in Table 4. Eighty-eight patients (74%) received a voriconazole-based first line therapy, either alone (n=49) or in combination with liposomal amphotericin B (n=20) or echinocandins (n=19). A total of 15 adverse events were recorded during voriconazole treatment (corresponding to 15% of the patients who received voriconazole overall), including four cases of hepatitis, four cases of peripherical neuropathy, three cases of

photosensitivity, three cases of periostitis, and one episode of visual hallucinations. These side-effects led to treatment interruption in 9 patients (60%).

Twenty-one patients underwent neurosurgery (two surgical interventions were performed in five patients and three in one) (Table 4). Comparison of clinical and radiological characteristic of operated and non-operated patients did not show any significant difference except for the median number of abscesses (1 [1-2.3] vs 4 [2-10] p=0.009) (table S5). The neurosurgical procedures included abscess resection (n=16) or drainage (*n*=2), lobectomy (*n*=2), ventricular shunting (*n*=7), and craniotomy/haematoma drainage (*n*=2). Ten patients (48%) experienced at least one complication of the surgical procedure: nosocomial meningitis (*n*=4), intracranial haematoma (*n*=2), oedema with or without intracranial hypertension (*n*=3), pseudomeningocele (*n*=1), and septic shock (*n*=1). None of the patients died following surgery. Ten patients underwent sinus surgery, including sphenoidectomy, ethmoidectomy, and middle meatal antrostomy.

Outcome

Mortality was 45% at 6 weeks (W6), 51% at 3 months, and 63% at 12 months (M12) (figure 3). W6 mortality was 57%, 73%, 41% and 32% in the HM, HSCT, SOT, and other diseases groups, respectively (table S2). W6 and M12 mortality varied significantly according to the route of dissemination (54% vs 19% at W6 in the haematogenous dissemination and contiguous groups, respectively, p<0.001 and 69% vs 46% at M12, p=0.04). The use of voriconazole as first-line treatment was associated with a better outcome at W6 (39% mortality at W6 in the voriconazole group vs 63% in others, p=0.02 but not at M12 (51% mortality vs 59% at M12, p=0.5). In univariate analysis, HM, neutropenia, haematogenous dissemination, shorter time to diagnosis, and the detection of GM in serum were associated

with a significantly lower six-week survival (table 5). After variable selection and correction for optimism, haematogenous dissemination, HM, and positive GM in the sera were predictive of W6 mortality.

Discussion

This large nationwide retrospective study provides new insights into the clinical, mycological, and radiological features of CA. We were able to characterize two different patterns of CA according to the presumed route of dissemination, reflecting several differences in brain infection pathophysiology.

Infection of the brain following haematogenous dissemination was seen in younger patients, most often with HM or SOT. In this population, GM and *Aspergillus* PCR were often positive in serum as well as in the CSF; the main radiological abnormalities were cerebral abscesses, often numerous and associated with small vessel disease. Progression of the disease was rapid, and associated with a high mortality. On the other hand, extension of a contiguous paranasal infection to the brain was seen in older and less immunosuppressed patients. GM and Aspergillus PCR were often negative in serum, but positive in the CSF. The main radiological abnormalities were a single abscess with frequent pachymeningeal involvement and large vessel disease, resulting in a high frequency of ischemic events. Progression of the disease appeared slower and associated with a lower mortality rate.

These observations have important implications for CA diagnosis, management and prognosis evaluation. Regarding diagnosis, two points should be emphasised. First, the low positivity rate of serum GM and *Aspergillus* PCR in patients with a contiguous infection suggests a limited release of *Aspergillus* DNA and cell wall components in blood. This is in accordance with the rarity of extracerebral aspergillosis in this group. Therefore, diagnosis of

this form of CA should not only rely on serum fungal biomarkers, but also on CSF biomarkers which should be measured in all suspected cases when feasible. Given the discordance observed between GM and PCR results in CSF, we recommend the determination of both. One should also bear in mind that a normal CSF analysis cannot be used to exclude the diagnosis of CA and is not correlated with GM or PCR result. Second, clinical examination was normal for almost 12% of the patients in our study. Based on a retrospective analysis of 29 children with CNS mold infection, the recent paediatric antifungal guidelines of ECIL-8 recommended performing CNS imaging for all children with IA irrespective of neurological symptoms (grade BII) (14,15). Our data further support brain MRI screening of IA patients to ensure the detection of CA as rapidly as possible.

The contiguous extension from a paranasal sinus infection was often associated with angioinvasion of proximal cerebral vessels, whereas hematogenous dissemination was associated with small vessel obstruction, possibly through an embolic process. Both result in a high frequency of ischemic lesions but with different topology. These observations raise questions about the potential benefit of anti-platelet aggregation therapy, particularly in patients with CA resulting from a contiguous sinus infection. A recent study of 41 cases of aspergillus carotidis highlighted the devastating consequences of carotid invasion in patients with localized cranial aspergillosis, with the occurrence of cerebral infarcts in 50% of the patients (16). Vascular imaging such as magnetic resonance image/magnetic resonance angiogram (MRI/MRA) should be performed in patients with CA to allow early detection and specific management of vascular abnormalities.

Finally, we identified HM and the detection of GM in the serum as the two independent prognostic factors for CA. This is clinically valuable because CA-related mortality remains as high as that reported more than 10 years ago (54-69%) (4,8). A lower mortality rate (38% at

M3) was recently reported, but the study included a limited number of patients, half of whom displayed lower levels of immunosuppression (6). In our study, mortality rates were particularly low for the patients who underwent surgery, but, given the retrospective design of the study, it would be risky to draw any firm conclusions from this observation. Indeed, the patients who did and did not undergo surgery were not comparable in terms of their underlying conditions and clinical status. Further studies will be needed to assess the potential impact of neurosurgery, its optimal timing and related morbidity in this fragile population.

This is the largest series of CA cases reported to date. This study has several strengths allowing for correlations and the identification of two distinct pathophysiological patterns. In contrast, its retrospective design entails inevitable limitations. Our conclusions are limited by the heterogeneity of diagnosis strategies and treatment, the non-availability of almost 30% of the imaging results used for diagnosis, as well as by partial information during follow-up (such as the evaluation of sequelae in survivors or the time course of changes in GM levels). Furthermore, this cohort is the result of screening mycological databases and physicians declarations, so it is vulnerable to recall bias which may alter its representativeness. Indeed, one could speculate that physician notifications of CA are predominantly found in cases in which the diagnosis is more obvious. Contrarily, cases which are difficult to diagnose are more likely to not result in any notification. This could result in a bias: only the evident cases are included, which favors the diagnostic tools that make the case so evident. Despite these limitations, however, our data provide physicians with useful insight into the diagnosis, management, and prognosis of CA.

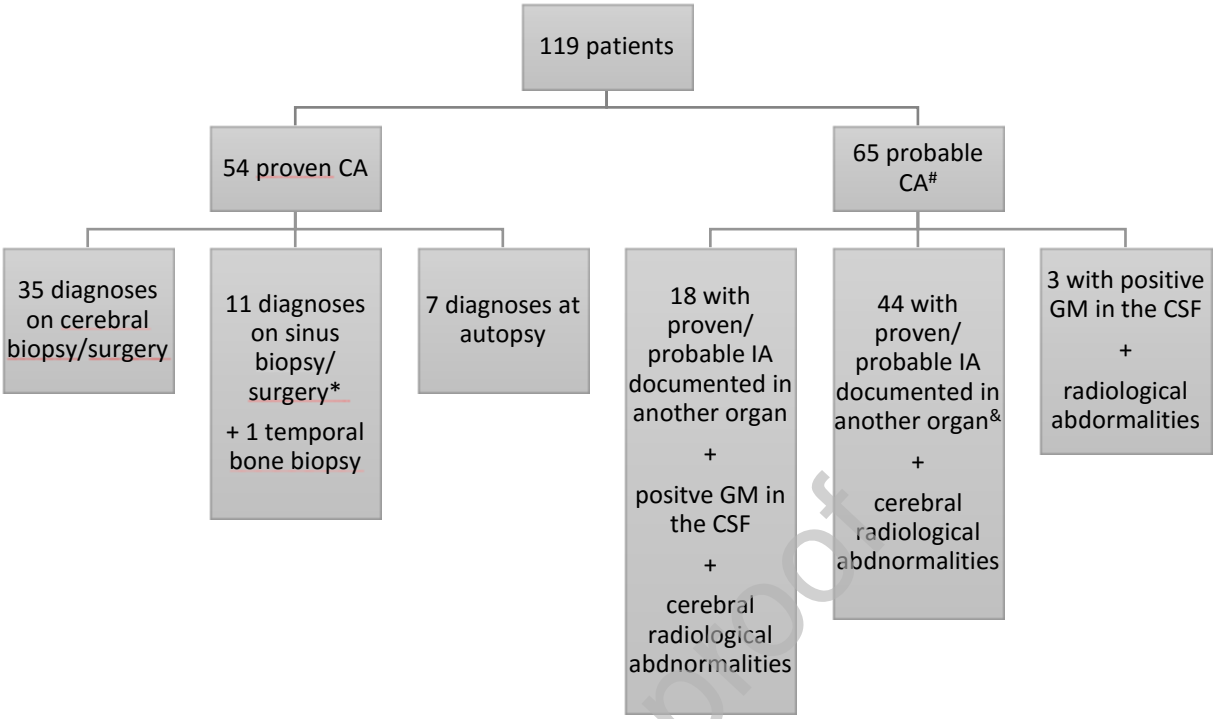


Figure 1. Flow chart of the mode of CA diagnosis

CA: cerebral aspergillosis; GM: galactomannan antigen

* diagnosis on sinus biopsy/surgery required: sinus biopsy with histological evidence of invasive mold infection associated with growth of *Aspergillus* following culture of the biopsied site and imaging showing a contiguous infection from the sinus to the brain.

[#] among the 65 probable CA, four patients were categorized as "modified probable CA" as diabetes mellitus was their only risk factor for IA.

[&] among the 44 probable CA without a positive dosage of GM in the CSF

- 12 patients had proven IA and 32 a probable IA in another organ
- Four patients had a positive *Aspergillus* PCR and one patient had a positive β-D-glucan in the CSF

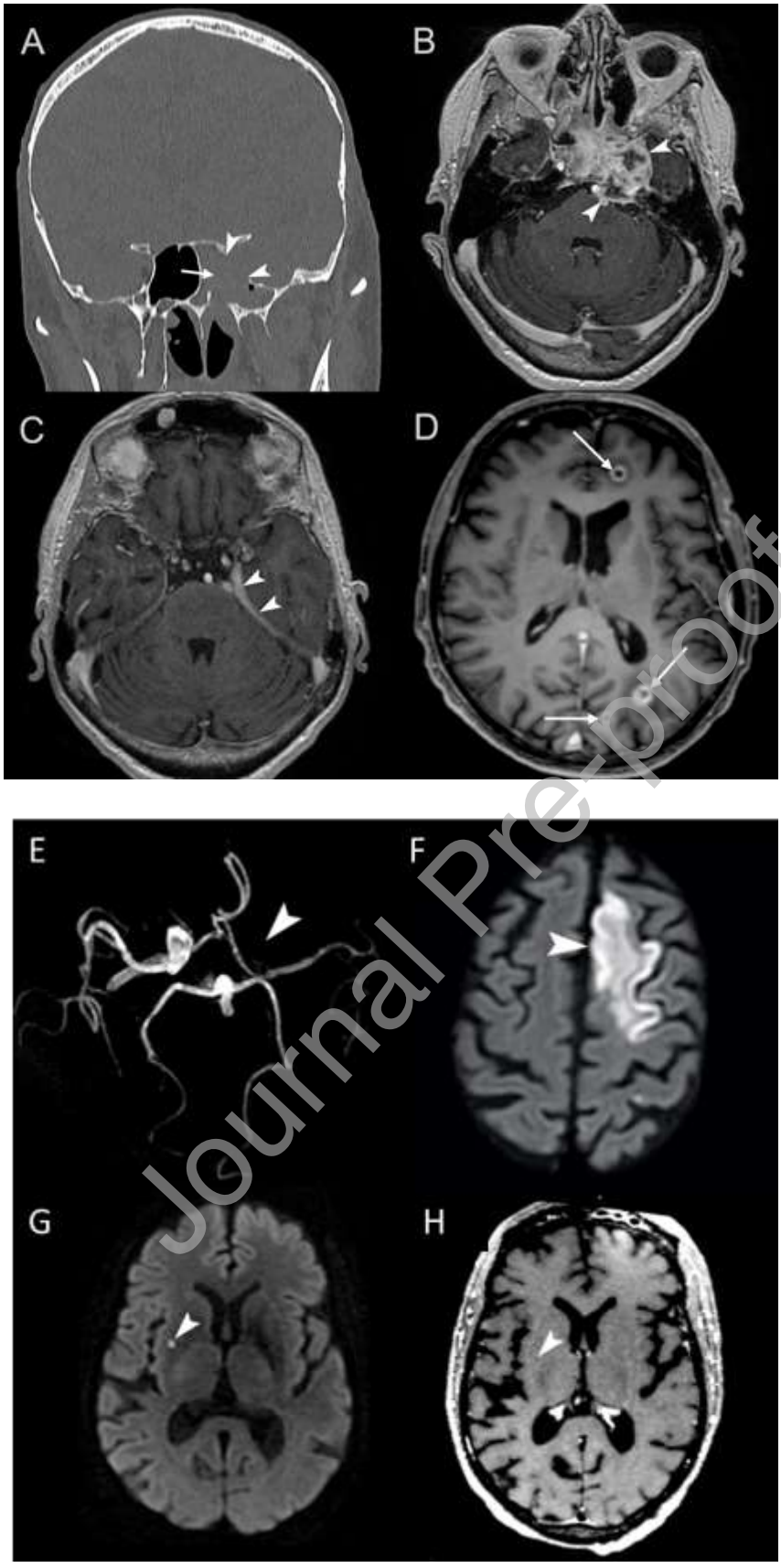


Figure 2. Examples of CNS imaging for patients with aspergillosis following contiguous (A, B, C) and haematogenous (D) spread with examples of macrovascular (E,F) and microvascular (G,H) lesions

A: Coronal CT view of the sphenoid sinus, showing left sphenoid aspergillosis (arrow) and osteolysis of the sphenoid wall (arrowheads).
B: Axial T1-weighted MRI sequence after contrast injection, showing a left sphenoid sinus mass extending into the CNS with a large extra-axial abscess (arrowheads) and left cavernous sinus involvement.
C: Axial T1-weighted MRI sequence after contrast injection, showing a thickening of the left cerebellar tentorium (arrowheads) corresponding to contiguous pachymeningitis
D: Axial T1-weighted MRI sequence after contrast injection, showing multiple annular contrast enhancing lesions (arrows) corresponding to haematogenic abscesses
E: Time-of-flight angiogram in axial view, showing an occlusion of the left carotid artery (arrowhead)
F: Corresponding diffusion-weighted image, showing a hyperintensity corresponding to a brain infarction systematised to the left anterior cerebral artery territory
G and H: Patient presenting a right lenticular lacunar infarct (arrowhead) on a diffusion-weighted sequence (C). The ischemic nature of the lesion is confirmed by the absence of enhancement on the corresponding post-contrast T1-weighted sequence (D)

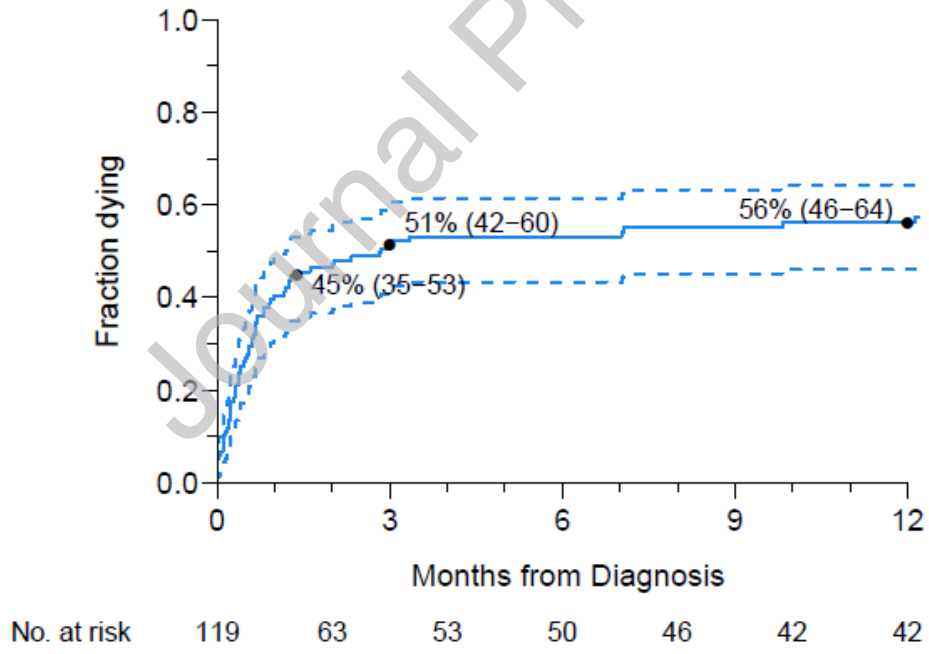


Figure 3. Overall mortality at 6 weeks, 3 months and 12 months
The dashed line indicates the pointwise 95% confidence interval. Three patients were lost to follow-up before three months and 14 were lost to follow-up before 12 months. The median follow-up of survivors was 16.8 months [9-36.7 months].

	Total (*n*=119)	Haematogenous dissemination (*n*=88)	Contiguous infection (*n*=31)	*p*-value
Age (years) at diagnosis, median and [IQR]	58 [47.5-66.5]	56 [44-62]	68 [55.5-77]	**<0.001**
Women, *n* (%)	40 (34%)	30/88 (34%)	10/31 (32%)	0.85
Main underlying diseases				
-Haematological malignancy[◊] *** HSCT** *** median time from HSCT to CA (months)**	47/119 (40%) 15 (13%) 3 [1-7.15]	39/88 (44%) 12 (14%) 4.0 [1.7-7.1]	8/31 (26%) 3 (10%) 0.4 [0.2-6.5]	0.07 0.3
-Solid organ transplant *** heart** *** lungs** *** kidney** *** liver** *** double transplant[¶]** **Median time from SOT to CA (months)**	34/119 (29%) * 13 (10.9%) * 3 (3%) * 11 (9%) * 5 (4%) * 2 (1.7%) 4.3 [2-7.5]	29/88 (33%) 13 (14.7%) 2 (2%) 8 (9%) 4 (5%) 2 (2%) 3.3 [1.8-7.4]	5/31 (16%) 0 (0%) 1 (3%) 3 (10%) 1 (3%) 0 (0%) 7.7 [6.2-17.8]	0.1 **0.03**
-Diabetes mellitus	10/119 (8%)	4/88 (5%)	6/31 (19%)	**0.02**
-Auto-immune disease	8/119 (7%)	6/88 (7%)	2/31 (6%)	1
-Other[§]	11/119 (9%)	7/88 (8%)	4/31 (13%)	0.5
Underlying disease				
Patients with 2 risk factors	23/119 (19%)	21/88 (24%)	2/31 (7%)	**0.04**
No underlying disease	9/119 (8%)	3/88 (3%)	6/31 (19%)	**0.009**
Immunosuppressive drugs[&] **- corticosteroids** **- T-cell immunosuppressive** *** ibrutinib**	 62/119 (52%) 85/119 (71%) 10/119 (8%)	 55/88 (63%) 69/88 (78%) 10/88 (11%)	 7/31 (23%) 16/31 (52%) 0/31 (0%)	 **<0.001** **0.004** 0.06
Neutropenia at diagnosis	18/119 (15%)	13/88 (15%)	5/31 (16%)	0.86
Lymphopenia at diagnosis	70/100 (70%)	54/71 (76%)	16/29 (55%)	**0.02**
Hypoalbuminemia	56/80 (70%)	45/60 (75%)	11/20 (55%)	0.23
Clinical signs				
Fever	48/119 (40%)	32/88 (36%)	16/31 (52%)	0.14
Headache	41/119 (34%)	21/88 (24%)	20/31 (65%)	**<0.001**
Impaired consciousness	67/119 (56%)	56/88 (64%)	11/31 (36%)	**0.01**
Seizure	25/119 (21%)	22/88 (25%)	3/31 (10%)	0.08
Focal sign	41/119 (34%)	33/88 (38%)	8/31 (26%)	0.2
Cranial nerve palsy	37/119 (31%)	17/88 (19%)	20/31 (65%)	**<0.001**
Normal neurological	14/119 (11.8%)	10/88 (11%)	4/31 (13%)	0.7

examination				
Median time between onset of symptoms and diagnosis (day) [IQR] (*n*=108):	4 [0-18]	2 [0-9]	54 [16-126]	**<0.001**
Extra-cerebral location of infection : - lung - sinus - skin - spleen - muscle - extracerebral vessel - kidney - bone - liver - endocarditis	104/119 (87%) 72 29 4 4 3 3 3 3 2 2	74/88 (84%) 71 0 4 4 3 2 3 2 2 2	30/31 (97%) 1[#] 29 0 0 0 1[#] 0 1[#] 0 0	0.07
CSF characteristics (67 lumbar punctures)				
High CSF protein levels (≥ 0.4 mg/mL)	39/52 (75%)	26/39 (67%)	13/13 (100%)	**0.03**
Meningitis (WBC>5/mm^3) (*n*=58)	25/58 (43%)	16/44 (36%)	9/14 (64%)	0.12
Median WBC count in cases of meningitis [IQR]	258 [70-885]	270 [41-872]	258 [80-885]	0.6
Predominance of polynuclear neutrophils in the CSF	19/23 (82%)	14/15 (93%)	5/8 (63%)	0.1
Outcome				
6-weeks mortality	53/118 (45%)	47/87 (54%)	6/31 (19%)	**<0.001**
12-month mortality	66/105 (63%)	54/79 (69%)	12/26 (46%)	**0.04**

Table 1. Clinical and biological characteristics of patients at diagnosis, by route of disease dissemination

HM : haematological malignancy; HSCT : haematologic stem cell transplant; SOT : solid organ transplant; CSF : cerebro-spinal fluid; WBC : white blood count

[◊] Haematological malignancy included acute leukaemia (13 cases of myeloid leukaemia and 7 of lymphoblastic leukaemia), chronic lymphoid leukaemia (13 patients), lymphoma (11 patients), and 3 other bone marrow disorders (one case of haemophagocytic syndrome, one of bone marrow primary aplasia, and one of myelodysplastic syndrome)

[¶] One patient had a heart and liver transplant and another had a lung transplant followed by a liver transplant

[§]other: HIV infection (*n*=2), post-surgery (*n*=3), cirrhosis (*n*=3), neoplasia (*n*=1), ARDS (*n*=1), primary immunodeficiency (*n*=1)

[&]received in the 6 months preceding CA

[#]all these extra-cerebral locations were reported in the same patient. The only extra-cerebral location reported in 29/31 patients of the contiguous group was the sinuses or the ears.

Of note, all patients under ibrutinib treatment had HM.

	Total (*n*=119)	Haematogenous dissemination (*n*=88)	Contiguous infection (*n*=31)	*p*-value
Positive culture*	86/119 (72%)	60/88 (69%)	26/31 (84%)	0.09
- *A. fumigatus*	76/86 (88%)	54/60 (90%)	22/26 (85%)	
- *A. flavus*	4/86 (5%)	0	4 (15%)	**0.004**
- *A. nidulans*	5/86 (6%)	5 (8%)	0	
- *A. terreus*	1 (1%)	1 (2%)	0	
- *Aspergillus.* sp	1 (1%)	1 (2%)	0	
Serum analysis				
Positive GM (>0.5)	66/104 (64%)	55/80 (69%)	11/24 (46%)	**0.04**
Median index of the positive GM	2.25 [1.05-4.7]	2.8 [1.1-4.99]	0.86 [0.57-1.37]	**0.003**
Positive β-D-glucan (>80 pg/ml)	15/18 (83%)	8/10 (80%)	7/8 (88%)	1
Median index of the positive β-D-glucan	431 [188->500]	431 [148->500]	377 [249.8->500]	0.94
Positive *Aspergillus* PCR	15/27 (56%)	15/22 (68%)	0/5 (0%)	**0.01**
CSF analysis (67 lumbar punctures)				
Positive GM (>0.5)	31/45 (69%)	25/34 (74%)	6/11 (55%)	0.28
Median index of the positive GM	5 [1.4-7.6]	4.9 [1.1-7.4]	5.6 [3.0-7.9]	0.37
Positive β-D-glucan	7/11 (64%)	4/7 (57%)	3/4 (75%)	1
Median index of the positive β-D-glucan	359.5 [194-475.5]	277.5 [151.5-426.5]	408.5 [362-454]	0.5
Positive *Aspergillus* PCR	14/26 (54%)	10/18 (56%)	4/8 (50%)	1
Positive for at least one biomarker	40/53 (76%)	32/40 (80%)	8/13 (62%)	0.26

Table 2. *Aspergillus* spp. identification and biomarkers, by route of disease dissemination

GM: galactomannan antigen, CSF: cerebrospinal fluid

* including all positive cultures from cerebral biopsies/surgeries, sinus biopsies, lung biopsies, bronchoalveolar liquid, skin, liver, muscle or arterial vessel biopsies

	Total (*n*=86)	Haematogenous dissemination (*n*=59)	Contiguous infection (*n*=23)	*p*-value
Number of imaging available for analysis :				
- MRI	68	49	19	
- CT-scan	18	14	4	
Abscesses	62/82 (76%)	56/59 (95%)	6/23 (26%)	**<0.001**
- number of abscesses				
*** 1**	19/62 (31%)	15 (27%)	4 (67%)	
*** 2-3**	14/62 (23%)	11 (20%)	2 (33%)	
*** 4-9**	15/62 (24%)	15 (27%)	0	
*** ≥10**	15/62 (24%)	15 (27%)	0	
Location of abscesses				
- supratentorial	40/62 (65%)	34/56 (60%)	6/6 (100%)	0.08
- infratentorial	4/62 (6%)	4/56 (7%)	0	
- both	18/62 (29%)	18/56 (32%)	0	
Median size of the largest abscess, mm [IQR]	21 [15-29.7]	21 [14-29.2]	24.5 [21.7-35.5]	0.3
Abscess wall enhancement	47/56 (84%)	41/50 (82%)	6/6 (100%)	0.6
Radiological meningitis	31/82 (38%)	12/59 (20%)	19/23 (83%)	**<0.001**
*** leptomeningitis**	11/82 (13%)	8/59 (14%)	3/23 (13%)	
*** pachymeningitis**	28/82 (34%)	7/59 (12%)	21/23 (91%)	
Ventriculitis	6/82 (7%)	3/59 (5%)	3/23 (13%)	0.34
Large vessels disease	19/53 (36%)	8/35 (23%)	11/18 (61%)	**0.01**
- arterial stenosis/occlusion	14/53 (26%)	4/35 (11%)	10/18 (56%)	
- cerebral ischemia	20/81 (25%)	12/59 (20%)	8/22 (36%)	
- aneurysm	1/53 (2%)	0/35 (0%)	1/18 (6%)	
Small vessel disease	31/74 (42%)	29/56 (52%)	2/18 (11%)	**0.002**
- haematoma	11/84 (13%)	11/62 (18%)	0/22 (0%)	
- micro-bleeding ≥5	9/59 (15%)	9/45 (20%)	0/14 (0%)	
- small subcortical infarct	21/67 (31%)	19/49 (39%)	2/18 (11%)	
Thrombophlebitis	3/81 (4%)	0/58 (0%)	3/23 (13%)	**0.02**
Cavernous sinus infiltration	7/81 (9%)	1/58 (2%)	6/23 (26%)	**0.001**

Table 3. Radiological characteristics, by route of disease dissemination

Neuroimaging was performed in all patients at diagnosis, but was available for analysis in 86 patients (72.3%): MRI for 68 patients and CT-scans for 18 patients.

	Total (n=119)	Haematogenous dissemination (n=88)	Contiguous infection (n=31)
Medical treatment			
Voriconazole	88/119 (73.9%)	54/88 (61%)	24/31 (77%)
• **alone**	49/119 (41%)	33/88 (38%)	16/31 (52%)
• **in dual therapy :**	39/119 (33%)	21/88 (24%)	8/31 (26%)
- **With L-Amb**	20/119 (17%)	12/88 (14%)	8/31 (26%)
- **With echinocandins**	19/119 (16%)	19/88 (22%)	0/31 (0%)
L-AmB	22/119 (18%)	15/88 (17%)	7/31 (23%)
• **alone**	15/119 (13%)	10/88 (11%)	5/31 (16%)
• **in dual therapy :**	7/119 (6%)	5/88 (6%)	2/31 (6%)
- **With echinocandins**	4/119 (3%)	3/88 (3%)	1/31 (3%)
- **With posaconazole**	2/119 (2%)	1/88 (1%)	1/31 (3%)
- **With 5FC**	1/119 (1%)	1/88 (1%)	0/31 (0%)
Caspofungine alone	2/119 (2%)	2/88 (2%)	0/31 (0%)
Unknown antifungal treatment	1/119 (1%)	1/88 (1%)	0/31 (0%)
Surgical treatment			
Neuro-surgery	21/119 (18%)	12/88 (14%)	9/31 (29%)
Complication following neuro-surgical procedures	10/21 (48%)	6/12 (50%)	4/9 (44%)

Table 4. Medical and surgical treatment received, by route of disease dissemination
NB : 6 patients did not receive anti-fungal therapy because the diagnosis was made post mortem

Variable	Univariate analysis	Multivariate analysis	Prediction model
Age at diagnosis (y)	1.00 (0.98 to 1.02)	1.01 (0.99 to 1.04)	—
Sex (male)	1.58 (0.72 to 3.51)	1.92 (0.78 to 4.72)	—
Haematogenous dissemination	4.89 (1.79 to 13.4)	3.62 (1.04 to 12.6)	2.44 (1.23 to 4.85)
Haematological malignancy	2.86 (1.04 to 7.85)	1.95 (0.61 to 6.25)	1.84 (1.06 to 3.19)
Solid organ transplant	1.48 (0.50 to 4.34)	0.84 (0.26 to 2.71)	—
Neutropenia	2.18 (0.76 to 6.20)	1.55 (0.41 to 5.91)	—
Time from 1st symptoms to diagnosis (weeks)	0.89 (0.80 to 0.98)	0.96 (0.88 to 1.05)	—
Positive GM in serum	2.84 (1.21 to 6.63)	2.15 (0.82 to 5.66)	2.07 (1.14 to 3.76)

Table 5. Factors associated with six-week mortality. The results presented are odds ratios and 95% confidence intervals. For the selected model, shrunken coefficients (corrected for optimism) are presented. All estimates are pooled over imputed datasets, data being missing for time from 1st symptoms to diagnosis (n = 11) and positive GM in serum (n = 15).

CEREALS article – supplementary material

Methods - Statistical analysis

Data are reported as counts and percentages for categorical data, and as the mean and standard deviation (SD) or median and interquartile range (IQR) for quantitative data. Follow-up was counted from the date of diagnosis to the date of death or last contact with the living patient. For patients diagnosed at autopsy, survival was set to zero. Overall survival was estimated by the Kaplan-Meier product-limit method. The primary outcome was three-month mortality. We accounted for patients for whom data were censored before three months of follow-up (which was the case for two patients), by weighting for the inverse probability of censoring (12). Missing baseline data were handled by multiple imputations, with chained equations, using the baseline cumulative hazard of failure in the imputation model (18). All variables considered for model development were used in the imputation model, together with the cohort, and the cumulative hazard (19). We generated and separately analysed 10 independent imputed datasets (20). The convergence of the multiple imputation algorithm was assessed by visual inspection of the mean and variance of the imputations streams. Estimates were then pooled over the 10 imputations according to Rubin's rules, to obtain point estimates and confidence intervals (CI) for each parameter. Logistic regression was used to estimate the association of a set of predefined variables (age at diagnosis, sex, underlying disease, neutropenia at diagnosis, time from first symptoms to diagnosis and positive GM in serum) and three-month mortality, after weighting to correct for censoring. For continuous variables, restricted cubic splines were used to display the shape of the relationship, and variables were then transformed. Given the small number of

events, we used three strategies for model development with the imputed data (21,22). We first used Wald tests for the pooled regression coefficients, to simplify the model with a backward selection procedure, with *p*-value cut-offs mimicking the use of the Akaike information criterion (AIC). We then used a similar backward elimination procedure on each imputed dataset and evaluated the proportion of imputed datasets in which the variables were selected. Finally, we used lasso regularisation to fit a final model, with a penalty factor determined by cross-validation. The lasso method has the advantage of selecting the model by shrinking coefficients to zero. Model performance was evaluated by calculating the concordance (*c*) statistic, as a measurement of discrimination, and Brier score, and from the calibration curve. The *c* statistic quantifies how well the model discriminates between the patients who die and those who survive, and corresponds to the area under the receiver operating characteristics (ROC) curve in the case of binary outcomes (23). It ranges from 0.5 to 1.0, with 1.0 indicating perfect discrimination. The Brier score is a measurement of overall performance. It ranges from 0 (perfect prediction) to 1 (poorest prediction). It is usually compared to the Brier score of the null model (without predictors), and should be lower. The calibration curve can be used to contrast the observed and predicted probabilities of an event, to evaluate the accuracy of model predictions. Performance was assessed by calculating the parameters for all the imputed dataset and then pooling (impute-last method), as previously recommended (24). Prognostic models derived from multivariable regression with variable selection are prone to overestimating regression coefficients; we therefore performed an internal validation of our model by bootstrapping (23). The second strategy for model selection was repeated in 200 bootstrap samples, and the model estimated from each bootstrap sample was then evaluated in the original sample. The differences between the performance on the bootstrap sample and that on the original

sample were used to assess the overoptimism of the selected model. The *c* statistic, corrected for overoptimism was then estimated, and the slope of the calibration curve was used as a shrinkage factor for the regression coefficients of the selected model. This shrinkage factor was compared with the shrinkage factor obtained by the lasso method. In each case, the predictions and the estimation of model performance were calculated for the imputed datasets and then pooled (impute-last method), as previously recommended (24). The final model is presented with the odds ratios obtained after shrinkage and their 95% confidence intervals (95% CI), together with a nomogram to facilitate the calculation of prediction score. All tests were two-tailed, and *P* values of 0.05 or below were considered to be statistically significant. Analyses were performed with R statistical software version 3.6.1.

Supplementary results

Comparison of sub-populations

	Non neutropenic patients (n=101)	**Neutropenic patients at diagnosis (n=18)**
Clinical signs		
Fever	35/101 (35%)	**13/18 (72%)**
Headache	34/101 (34%)	7/18 (39%)
Impaired consciousness	56/101 (55%)	11/18 (61%)
Seizure	20/101 (20%)	5/18 (28%)
Focal sign	34/101 (34%)	7/18 (39%)
Cranial nerve palsy	28/101 (28%)	9/18 (50%)
Normal neurological examination	13/101 (13%)	1/18 (6%)
Microbiological characteristics		
Biological meningitis (WBC>5/mm3)	24/49 (49%)	1/9 (11%)
Positive GM in the sera	51/86 (59%)	15/18 (83%)
Radiological characteristics		
Abscesses	55/71 (78%)	7/11 (64%)
Contrast enhancement	42/50 (84%)	5/6 (83%)

Meningitis	27/71 (38%)	4/11 (36%)
Ventriculitis	5/71 (7%)	1/11 (9%)
Macro-angiopathy	16/47 (34%)	3/6 (50%)
Micro-angiopathy	28/64 (44%)	3/10 (30%)
Mortality		
Mortality at 6 weeks	42/100 (42%)	11/18 (61%)
Mortality at 12 months	53/90 (59%)	13/15 (87%)

Table S1. Comparison of clinical and radiological characteristics of neutropenic versus non neutropenic patients at diagnosis

GM: galactomannan antigen, WBC: white blood count

	HM (n=47)	**SOT (n=34)**	**Other (n=38)**
Age (years) at diagnosis, median and [IQR]	61 [44.5-67]	58 [44.5-67]	57 [48-72.8]
Number of Female (%)	19 (40%)	11 (32%)	10 (26%)
Hematogenous dissemination	39/47 (83%)	29/34 (85%)	20/38 (53%)
Clinical signs			
Fever	**26/47 (55%)**	**5/34 (15%)**	**17/38 (45%)**
Headache	15/47 (32%)	9/34 (27%)	17/38 (45%)
Impaired consciousness	**16/47 (34%)**	**22/34 (65%)**	19/38 (50%)
Seizure	11/47 (23%)	6/34 (18%)	8/38 (21%)
Focal sign	21/47 (45%)	10/34 (29%)	10/38 (26%)
Cranial nerve palsy	12/47 (26%)	12/34 (35%)	13/38 (34%)
Normal neurological examination	6/47 (13%)	4/34 (12%)	4/38 (11%)
Biological characteristics			
Neutropenia at diagnosis	16/47 (34%)	1/34 (3%)	1/38 (2.6%)
Lymphopenia at diagnosis (n=100)	27/39 (69%)	27/31 (87%)	16/30 (50%)
Hypoalbuminemia (n=80)	20/32 (63%)	19/25 (76%)	17/23 (74%)
Biological meningitis (WBC>5/mm3) (n=58)	10/26 (39%)	8/18 (44%)	7/14 (50%)
Positive GM in the sera (n=104)	25/42 (60%)	24/31 (75%)	17/31 (55%)
Radiological characteristics			
Abscesses	22/28 (79%)	25/29 (86%)	15/25 (60%)
Contrast enhancement	15/20 (75%)	19/22 (86%)	13/14 (93%)
Meningitis	8/28 (29%)	9/29 (31%)	14/25 (56%)
Ventriculitis	2/28 (7%)	3/29 (10%)	1/25 (4%)

Macro-angiopathy	5/18 (28%)	7/17 (41%)	7/18 (39%)
Micro-angiopathy	9/27 (33%)	16/30 (53%)	6/17 (41%)
Mortality			
Mortality at 6 weeks	27/47 (58%)	14/34 (41%)	12/37 (30%)
Mortality at 12 months	30/41 (73%)	17/31 (55%)	19/33 (58%)

Table S2. Comparison of clinical and radiological characteristics of the patients according to their underlying diseases :

HM: haematological malignancy , SOT: solid organ transplant , GM: galactomannan antigen

Other includes : diabetes (n=10), autoimmune diseases (n=8), HIV infection (*n*=2), post-surgery (*n*=3), cirrhosis (*n*=3), neoplasia (*n*=1), ARDS (*n*=1), primary immunodeficiency (*n*=1) and no underlying disease (n=9)

	HM	**SOT**	**Diabetes**	**Auto-immune diseases**	**Other risk factors**	**No risk factor**	**total**
Serum GM positivity	25/42 (59.5%)	24/31 (77.4%)	2/8 (25%)	5/7 (71.4%)	5/8 (62.5%)	5/9 (55.5%)	66/104 (63.5%)

Table S3. Galactomannan positivity at diagnosis according to the underlying disease

HM: haematological malignancy , SOT: solid organ transplant , GM: galactomannan antigen

Concomitant infections

A systemic bacterial co-infection was diagnosed in 15 (12.6%) patients, predominantly involving Gram-negative bacteria (4 among the proven CA and 11 among the probable CA).

A invasive fungal concomitant infection was diagnosed in 13 patients (10.9%) (including 4 with a proven CA and 9 with a probable CA). Twelve of the 13 patients were in the HM and SOT groups (12/13). The co-infection was documented in the brain in only one patient (Mucorales). Ten patients (8.4%) had CMV reactivation, but none displayed any evidence of CMV disease.

	Fungus identified	Localization of the concomitant fungal infection	Diagnosis method of CA	Outcome
Patient 1	*Pneumocystis*	Lungs	proven	Alive at S6
Patient 2	*Mucorales*	Kidney	proven	Alive at S6
Patient 3	*Candida (C. albicans)*	Blood and ascites	probable	Deceased
Patient 4	*Candida (C. krusei)*	Blood	probable	Deceased
Patient 5	*Candida (C. krusei)*	Blood	probable	Deceased
Patient 6	*Candida sp*	Peritoneum	probable	Alive at S6
Patient 7	*Pneumocystis*	Lungs	proven	Alive at S6
Patient 8	*Trichosporon mycotoxinivorans* and *Scedosporium apiospermum*	T : blood, heart and lungs S : lungs, heart, liver, intestine, kidney	probable	Deceased
Patient 9	*Candida (C. albicans)*	Blood	probable	Deceased
Patient 10	*Mucorales (Rhizomucor)*	Brain	proven	Deceased
Patient 11	*Candida (C.krusei)*	Blood	probable	Deceased
Patient 12	*Mucorales (rhizomucor)*	Pleura	probable	Deceased
Patient 13	*Mucorales (Rhizopus)*	Lungs	probable	Deceased

Table S4. Fungal co-infections and associated outcome.

Treatment

Twenty-one patient underwent neuro-surgery. Main characteristics of operated on patients are displayed in table S5.

	Surgery (*n*=21)	**No surgery (*n*=98)**
Median age (years)	54 [39-62]	59 [48.25-67]
Underlying context		
- HM	6/21 (29%)	41/98 (42%)
- SOT	4/21 (19%)	30/98 (31%)
- Other	11 /21(52%)	27/98 (28%)
Haematogenous dissemination	12/21 (57%)	76/98 (78%)[◊]
Neutropenia at diagnosis	1/21 (5%)	17/98 (17%)
Radiological findings:		
- abscesses	12/12 (100%)	50/70 (71%)
*** median number of abscesses**	1 [1-2.3]	4 [2-10][§]
*** size of the largest abscess**	24 mm [19-31]	21 mm [14-29]
- meningitis	6/12 (50%)	25/70 (36%)
- microangiopathy	3/10 (30%)	28/64 (44%)
- macroangiopathy	0/7 (0%)	19/46 (41%)
6-week mortality	2/21 (10%)	51/97 (53%)

Table S5. Characteristics of the patients who underwent surgical procedures

◊ p=0.053

§ p=0.009

HM: haematological malignancy, SOT: solid organ transplant

Outcome

The six-week 6 mortality was 15/55 (27.3%) and 38/64 (59.3%) for proven and probable CA, respectively. In most studies IFI proven cases mortality is higher than probable cases. In our study, several particularities may explain this result. Diagnosis of proven CA requested either a cerebral biopsy or a sinus biopsy (with a contiguous infection): the most critically ill patients, or the one who died rapidly didn't have a cerebral biopsy; sinus biopsy meant a contiguous infection of the brain from a paranasal sinus, which is associated with a better outcome.

We divided the inclusion period into 3 equally-sized categories (with cut points at November, 2012 and June, 2015). Adjusted for the final model, W6 mortality did not change significantly over the study period :

- Odd ratio of 0.90 (0.44 to 1.84) for period 2 (between Nov 2012 and June 2015) compared to 1 (before Nov 2012)
- Odd ratio of 0.85 (0.40 to 1.77) for period 3 (after June 2015) compared to 1 (before Nov 2012)

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Author's contribution
FL and OL coordinated the work of the authors and guided the development of the study. AS and FD collected the cases, and wrote the initial manuscript draft. JB and ON reviewed all brain imaging. Statistical analyses were performed by AS and RP. Other authors (RS, MW, SK, VL, AF, GH, VP, M-EB,SP, FA, FP, FC, PT, J-PG,LL, SC, FB, SH, SB, RH) critically revised the draft and added important intellectual content. All authors participated in review and revisions, approved the final manuscript and are accountable for all aspects of the work and for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors had access to and verified the data here presented.

Conflict of interest disclosure
Dr Serris, Dr J. Benzakoun, Dr R. Porcher, Dr G. Hekimian, Dr L. Lelièvre, Dr F. Bonneville, Dr M-E. Bougnoux, Dr S. Houze, Dr S. Bretagne, Dr S Kremer, Dr V Letscher-Bru, Dr V. Pourcher, Dr S. Poirée, Dr FA, Dr P. Tattevin have nothing to declare.
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