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Original article

Altered thymic CD4$^+$ T-cell recovery after allogeneic hematopoietic stem cell transplantation is critical for nocardiosis

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ABSTRACT

Purpose of the study: Nocardia affects immunocompromised human host exhibiting an altered cell-mediated immunity. Infectious risk after allogeneic hematopoietic cell transplantation (AHCT) is significantly correlated to the recovery status of donor-derived immune system, especially CD4$^+$ T-cells reconstitution and thymopoiesis. The purpose of this paper is to highlight a lack of cell-mediated immunity recovery for patients presenting a nocardiosis compared to a control cohort.

Patients and methods: This is a case control retrospective monocentric study. We retrospectively analyzed a monocentric cohort of 15 cases of nocardiosis after AHCT and we explored the degree of patients' immunosuppression by phenotyping circulating lymphoid subpopulations, including NK cells, CD8$^+$ T-cells, CD4$^+$ T-cells and CD19$^+$ B-cells. We focused on CD4$^+$ T-cell subsets to appreciate thymic output, especially on naive CD4$^+$ T-cells (NTE, CD45RA$^+$/RO$^-$ CD4$^+$ T-cells) and recent thymic emigrants (RTE, CD4$^+$CD45RA$^+$/RO$^-$/CD31$^+$). Infected patients were paired with a control cohort of patients with identical transplantation characteristics screened on hematological disease, AHCT conditioning, primary graft-*versus*-host disease (GHVD) prophylaxis, graft type, sex, age, and season at the AHCT and data concerning immunological reconstitution were compared.

Results: At onset of nocardiosis, circulating lymphocytes and CD4$^+$ T-cells means count were respectively 730/μL and 162/μL. CD8$^+$ T-cells, CD56$^+$ NK cells and CD19$^+$ B-cells means count were respectively 362/μL, 160/μL, 112/μL. CD4$^+$ T-cells subpopulations, naïve CD4$^+$ T-cells production was impaired with NTE and RTE means count at 26/μL and 11/μL respectively. Comparison between nocardiosis cohort and control cohort over time highlight significant lower cellular count for lymphocytes, CD4$^+$ T-cells, NTE and RTE with $p = 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively.

Conclusion: Immune recovery monitoring follow-up after AHCT is of particular importance to identify patients susceptible to develop Nocardiosis. Efficient microbiological investigations toward *Nocardia* such PCR should be used in case of compatible clinical presentation.

Introduction

Nocardia is an ubiquitous group of environmental bacteria belonging to the suborder of aerobic Actinomycetes with *Rhodococus* within the family *Nocardiaceae*, close to the genus *Mycobacterium*. This is an aerobic gram-positive filamentous bacterium with hyphae-like branching. *Nocardia* are saprophytic organisms that can be found in soil, decomposing vegetation, organic matter from animal, as well as water and dust. Several heterogeneous species were described with numerous recent taxonomy revisions [1,2].

Nocardiosis is the name of *Nocardia spp* infection, and could affect immunocompromised human hosts [3]. This opportunistic pathogen affects particularly patients exhibiting an altered cell-mediated immunity [4]. Solid-organ and hematopoietic stem cell transplant recipients concerned one third of nocardiosis [2,5]. The clinical presentation is heterogeneous, non-specific and depends on infectious sites [4]. The most common infectious site is lung by respiratory tract infestation [6], nevertheless other localizations

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with disseminated presentation could be presented in blood, central nervous system (CNS) or lymph nodes [7]. The common radiological presentation does not have pathognomonic features as it shows lungs condensation with abscesses and nodules. CNS dissemination is also usually described with parenchymal abscesses [2,5,7,8]. As rare and late after allogeneic hematopoietic cell transplantation (AHCT), nocardiosis is potentially lethal with a survival rate reaching 84% (in retrospective studies) [9]. Gathering cohorts of immunosuppressed patients, incidence is estimated from 0.3 to 3.6% [2,9]. This incidence is likely under-estimated [5] as result of *Nocardia* identification challenge [10].

Despite improvement of supportive care during the last decades, infections are still the second cause of mortality after allogeneic hematopoietic stem cell transplantation (AHCT) [11,12]. The infectious risk after AHCT is significantly correlated to the delayed recovery of donor-derived immune system, especially $CD4^+$ T-cells subset expansion [13–15]. Long-lasting steroids and immunosuppressive drugs use in AHCT impair immune recovery and are associated to nocardiosis presentation [2,9,10,16]. We report here a monocentric cohort of patients who underwent AHCT and presented a nocardiosis. The case control analysis depicts the defect of cell-mediated immunity recovery associated with this opportunistic infection.

Patients and methods

We described nocardiosis characteristics, management and outcome after AHCT in a hematopoietic cell transplant recipients' cohort treated at the University Hospital of Besançon. We analyzed immune reconstitution through circulating T-cells populations that were phenotyped before, at onset and after nocardiosis. The different circulating lymphoid populations available for analysis included: NK cells, $CD8^+$ T-cells, $CD4^+$ T-cells and $CD19^+$ B-cells. Our regular panel assessing immune reconstitution focused more precisely on naive thymic emigrant $CD4^+$ T-cells (NTE identified as $CD45RA^+/RO^-$) and recent thymic emigrants CD4+ T-cells (RTE identified as $CD45RA^+/RO^-/CD31^+$) to appreciate thymic output impact after transplantation.

Patients

Patients were selected from the local clinical data base between May 2001 and December 2017. We included all nocardiosis cases defined as "proven", "probable" or "possible" based on the similar invasive fungal infection (IFI) classification [17]. We defined "possible" nocardiosis by the presence of clinical and radiological features (nodule, condensation, abscess), with or without dissemination [8], no alternative diagnosis and improvement after anti-*Nocardia* treatment in an immunocompromised host. "Probable" nocardia was defined by identification of non-direct bacteriological criteria (direct exam, pathology, positive *Nocardia* PCR), and "proven" by positive *Nocardia* culture confirmation, as described recently [18] (Table 1). Pathology arguments to bacterial infection or gram-positive bacillus were considered as non-direct bacteriological criteria.

In purpose to strength our analysis, we selected a matched 2:1 control cohort based on the following matching criteria: on hematological disease, AHCT conditioning (myeloablative, MAC or reduced intensity conditioning regimens, RIC), primary graft-*versus*-host disease (GHVD) prophylaxis (drugs regimens), HLA matching (MRD, MUD, MMUD and haploidentical), sex, age, and season at the AHCT from retrospective data base. Immune reconstitution data over time were compared between these two groups during the first two years after AHCT.

Table 1
Nocardiosis definitions.

Possible	Compatible host + clinic and radiologic criteria
Probable	Possible + non-direct microbiologic criteria (direct exam, pathology, PCR)
Proved	Previous criteria + positive culture

Data were collected retrospectively. This non-interventional study was conducted under the French Medical Research Data Processing Advisory Committee (CCTIRS) and the French Information Technology and Privacy Commission (CNIL) recommendations about anonymous clinical data use. All patients sign a consent form permitting to use their clinicals data anonymously respecting the Helsinski declaration.

We collected data concerning hematological disease, AHCT management, follow-up of immune recovery, and nocardiosis characteristics directly from the charts.

Statistic methods

Statistical analyses and graphs were performed with Rstudio® Version 1.1.463. Continuous variables were summarized with means and standard deviation with interval of confidence at 95%. All observations over time were represented on stripcharts with identification of median value. Immune recovery was evaluated at least every three months during the first 2 years (more frequently for frail patients with GVHD or recurrent infection) and compared with the control population. Patients were censured at death time. Means of T-cell subset count were compared by Mann–Whitney test.

AHCT management

Conditioning and GVHD prophylaxis were adapted to hematological disease and comorbidity score, such as updated Sorror's score [19], as well as to our local policy and European Bone Marrow Transplantation Group (EBMT) recommendations.

During the first month after AHCT, all patients were hospitalized in High Efficiency Particulate Air laminar flow chamber until neutrophilic recovery. An intravenous antibiotic therapy was administered empirically or adjusted to patient's environment, in case of fever [20]. Bacterial, viral and fungal prophylaxis were administered as already described: long-term valaciclovir [21], fluconazole or posaconazole during 6 months or more in case of GVHD [22,23], trimethoprim-sulfamethoxazole (TMP-SMX 800 mg/160 mg) three time a week, or in case of adverse event atovaquone or aerosol of pentamidine [24]. Intravenous polyvalent immunoglobulins were administrated (400 mg/kg) in case of immunoglobulin G <3 g/L. Vaccines were administered according to EBMT schedules [25] and adapted to the immune recovery.

Cytomegalovirus (CMV) PCR and fungal biomarkers (galactomannan (GM) completed by specific *Aspergillus* PCRs) monitoring were performed once a week until immunosuppressive therapy withdrawal [26]. In case of sepsis the following tests were systematically performed: blood cultures, urine bacteriological analysis, GM, *Aspergillus*, *Mucormycosis* [27], *Toxoplasma gondii*, and CMV, C-reactive protein count, thoracic CT scan, and also *Pneumococcus* and *Legionella* antigenuria in case of pulmonary infection. In case of lack of microbiological documentation by non-invasive approach, *Nocardia* detection was included in our pneumological investigational protocol for immunocompromised patients by bronchial-alveolar lavage (BAL). *Nocardia* detection was also realized in case of abscess after biopsy and association of clinical and radiological presentation compatible with nocardiosis [8]. In case of nocardiosis diagnosis, a cerebral evaluation was systematically performed by magnetic resonance imaging (MRI) or by CT scan if MRI was not rapidly available.

Specific microbiological detection of Nocardia

All bacteriological samples were initially observed by Gram direct examination. Then they were usually seeded in standard bacteria growth media with daily examination for growth. In this kind of regular growth media, *Nocardia* species grow slowly, until 2–3 weeks [10]. *Nocardia* colonies appeared chalky white in case of *hyphae* production.

Nocardia confirmation was centralized to the referent laboratory in Lyon (*Observatoire français des nocardioses*), and was identified by PCR as recently published [18]. Antimicrobial agent sensibility was tested in vitro on isolates.

Immune recovery assessment

Lymphocytes counts were evaluated by XN-10 system automat (Sysmex, Japan). Absolute lymphocytes, $CD4^+$ T-cells, $CD8^+$ T-cells, $CD56^+$ NK cells and $CD19^+$ B-cells counts were determined by flow cytometry by TetraCXP1 method and fluorescence evaluation by FC500 cytometer (Beckman Coulter, France) [28]. Naïve $CD4^+$ T-cells population was characterized by $CD45RA^+/CD45RO-/CD4^+$ labeling in immunofluorescence. $CD31^+$ expression was evaluated in order to identify RTE lymphocytes and subsequent thymic lymphocyte maturation [29]. Our laboratory benchmark for general healthy population was 493–1666 $CD4^+$ T-cells/μL, 224–1112 $CD8^+$ T-cells/μL, 75–520 $CD19^+$ B-cells/μL and 73–654 $CD56^+$ NK cells/μL. Quantitative immunoglobulin evaluation was also performed to quantitively assess the humoral immune recovery.

Chimerism was performed on bone marrow or on blood by semi-quantitative PCR with unique donor and recipient short tandem repeat markers (STR) amplification [30]. The quantification was obtained by donor and recipient STR loci fluorescence GeneScan computer analyze (Applied Biosystem, France).

Results

Population

Between May 2001 and December 2017, we highlighted 15 cases of nocardiosis in the 752 AHCT realized in our hematological department, incidence rate of 1.99%.

In the nocardiosis cohort one patient received 2 AHCT, acute myeloid leukemia (AML) was the most frequent hematological disease (61%), 10 patients (63%) were in complete response (CR) at the transplantation time, 2 in partial response, 3 in progressive disease. Males were preponderant (sex ratio 4:1) (Table 2). Myeloablative conditioning regimens (MAC) were used for 9 patients (60%) and the 6 others conditioning regimens (40%) were reduce intensity conditionings (RIC). Ten patients (77%) received anti-thymoglobulin serum for GVHD prophylaxis during conditioning regimen (2.5 mg/kg for 1 patient, 5 mg/kg for 8 and 7.5 mg/kg for one). Median $CD34^+$ infused cells count was 2.69×10^6/kg [IC 95%, 1.85–3.57]. Characteristics were similar to control cohort as report in Table 2.

Nocardiosis presentation

Six nocardiosis occurred in patient receiving AHCT in spring whereas others were equally presented in summer, autumn and winter. Median duration time between AHCT and nocardiosis diagnosis was 218 days [IC95%, 118–317]. On the 15 nocardiosis, 7 (47%) were localized then 8 (53%) were disseminated (at least 2 infectious sites). Thirteen (87%) presented a pulmonary involvement, 8 (53%) were neurological nocardiosis with central nervous system (CNS) involvement with 4 (24%) ocular involvement, 2 (12%) were skin nocardiosis and 1 was a bacteriema (Table 3 and 4).

Table 2
Transplant characteristics.

	Nocardiosis cohort	Control cohort
Mean age (years) (p = 0.11)	49 [43–55]	43 [37–49]
Sex ratio	4:1	4:1
Hematological disease		
Acute myeloid leukemia	9 (61%)	18 (61%)
Acute lymphoblastic leukemia	2 (13%)	4 (13%)
Myelodysplasia	2 (13%)	4 (13%)
Multiple myeloma	2 (13%)	4 (13%)
Conditioning		
Myeloablative conditioning	9 (60%)	18 (60%)
Reduce intensity conditioning	6 (40%)	12 (40%)
HSCT type		
Bone marrow	8 (54%)	16 (54%)
Peripheral blood stem cells	7 (46%)	14 (46%)
Donor type		
Sibling	8 (54%)	16 (54%]
Unrelated donor	4 (27%)	10 (33%)
9/10	1 (6%)	–
Haplo identical	2 (13%)	4 (13%)
CMV donor/recipient status		
+/+	4 (27%)	11 (37%)
+/−	3 (20%)	2 (6%)
−/+	5 (33%)	9 (30%)
−/−	3 (20%)	8 (27%)
Graft-versus-host disease		
Acute grade	12 (80%)	21 (70%)
Grade I–II	8 (53%)	15 (50%)
Grade III–IV	4 (27%)	6 (20%)
Chronic	11 (73%)	21 (70%)
Mild	1 (6%)	10 (33%)
Moderate	7 (47%)	7 (24%)
Severe	3 (20%)	4 (13%)
Steroid cumulative dose		
Mean during 1st month (p = 0.20)	25 mg/kg/4weeks [15.5–34.9]	19 mg/kg/4weeks [8.5–28.6]

Table 3
Nocardiosis characteristics (n = 15).

Prophylaxis (TMP-SMX)	8 (53%)	Antibiotic treatment (n = 17)	
		Induction	
Infection status		Ceftriaxone	1 (6%)
Localized	7 (47%)	Ceftriaxone + Linezolid	1 (6%)
Disseminated	8 (53%)	IMP	5 (29%)
		IMP + AMK	3 (18%)
Infection site		IMP + TMP-SMX	2 (12%)
Pulmonary	13 (87%)	IMP + Linezolid	1 (6%)
CNS	8 (53%)	MERO + Linezolid	2 (12%)
Ocular	4 (27%)	MERO + Linezolid + AMK	1 (6%)
Cutaneous	2 (12%)	Cefuroxim + TMP-SMX	1 (6%)
Bacteremia	1 (6%)		
		Consolidation	
Documentation		TMP-SMX	4 (24%)
Cyto-histopathology	3 (20%)	TMP-SMX + Cefuroxim	3 (18%)
PCR	10 (66%)	TMP-SMX + Ceftriaxone	1 (6%)
Culture	5 (66%)	TMP-SMX + Cefotaxim	1 (6%)
		Moxifloxacin	2 (12%)
Co-infection		Ceftriaxone	2 (12%)
CMV	4 (26%)	MERO + Linezolid	1 (6%)
Pneumocystis	2 (13%)	Minocycline + Ciprofloxacin	1 (6%)
Mucormycosis	2 (13%)	Dead before	2 (12%)

Mean steroid dose at diagnosis 0.30 mg/kg/day [0.19–0.58].
IMP imipenem, MERO meropenem, AMK amikacin, TMP-SMX trimethoprim-sulfamethoxazole, CNS Central Nervous System.

Main radiological presentation was abscesses (11/15, 73%), with lungs nodules (7/13, 53%) and condensation (9/13, 69%) half time associated to abscesses (5/13, 38%), and CNS abscesses (8/8). As previously defined, 5 nocardiosis cases were proved, 6 were probable, 4 were possible (Table 4). Ten (66%) were documented by PCR, 3 (20%) by cyto-histopathology, 5 (33%) by late culture with 1

Table 4
Nocardiosis details.

Patient	Prophylaxis	Infectious site	Radiology	Sample site	Pathology	Culture	PCR	Species	Status
#1	TMP-SMX	Lung	Chest CT scan: lung abscess	Lung biopsy	+	+	+	*N. cyriacigeorgica*	Proved
#2	None	Skin	Smooth tissue CT scan: abscess	Skin biopsy	–	+	+	*N. farcinica*	Proved
#3	TMP-SMX	CNS, Oc, Lung	Cerebral MRI: multiple abcesses	Lung biopsy	indirect	+	+	*N. farcinica*	Proved
#4	TMP-SMX	CNS, Oc, Lung	Cerebral CT scan : multiple abcesses + Chest CT scan : lung nodule	BAL	–	not made	+	*N. nova*	Probable
#5	TMP-SMX	CNS, Oc, Lung	Cerebral CT scan : multiple abcesses + Chest CT scan : lung nodule	CSF, vitreous, BAL	indirect	–	–	–	Possible
#6	TMP-SMX	Lung, pleural	Chest CT scan : lung nodule + pleural condensation	Lung biopsy	+	not made	+	*N. shimofuensis / higoensis*	Probable
#7	Pentamidine	CNS, Oc, Lung	Cerebral CT scan : multiple abcesses + Chest CT scan : small lung nodule + pleural condensation	BAL	not made	–	+	N. nova	Probable
#8	Pentamidine	CNS, Lung	Cerebral MRI : 2 large abcesses + Chest CT scan : lung nodule + Chest CT scan : lung abscess + lungs nodules	CSF, BAL, Abscess	+	+ (abscess)	+ (CSF)	*N. nova*	Proved
#9	TMP-SMX	CNS, Lung	Cerebral MRI : abcesses	No-biopsy able, BAL not made	not made	not made	not made	–	Possible
#10	Atovaquone	Lung	Chest CT scan : lungs condensations	BAL not made	not made	not made	not made	–	Possible
#11	TMP-SMX	CNS, Lung, Skin	Cerebral CT scan : abcesses + Body CT scan : lungs condensations + liver abscess	Blood culture	not made	+	not made	N. farcinica	Proved
#12	TMP-SMX	Lung	Chest CT scan : lungs condensations + lungs nodules	BAL	indirect	Few GPB, none identification able	+	*N. abscessus*	Probable
#13	None	Lung	Chest CT scan : lungs nodules, condensation, abcesses + pleural condensation	Pleural biopsy	indirect	–	+	N. farcinica	Probable
#14	None	CNS	Cerebral CT scan : compressive abcess	CSF	–	–	+	*N. spp*	Probable
#15	None	Lung	Chest CT scan : lung abcess + pleural condensations	BAL No-biopsy able	–	–	not made	–	Possible

CNS : central nervous system, Oc : ocular, CSF : cerebrospinal fluid, BAL : bronchial alveolar lavage, GPB : gram-positive bacillus.

(6%) on blood culture. Five patients had nocardia detection through at least 2 different methods. Four patients had missing documentation: 3 without possible laboratory *Nocardia* detection on samples and one which the sample was lost. Nevertheless they were highly suspected based on clinical and radiology presentation and because of the negative regular microbiological investigation regard and the favorable evolution under specific anti-*Nocardia* treatment.

Two patients presented a nocardiosis recurrence: one after a second AHCT 3 years later and the other one 2 months after antimicrobial prophylaxis ending. For this patient, the secondary prophylaxis had been maintained Eight patients received TMP-SMX prophylaxis at nocardiosis diagnosis and relapse. Among them, 3 had gut GVHD. Four patients presented concomitant CMV reactivation and 4 other patients presented co-infection (2 *Mucormycosis* and 2 *Pneumocystis*). One of the patients with pneumocystosis did not followed the prescribed TMP-SMX prophylaxis. Median duration time of antimicrobial treatment was 180 days [IC95%, 130–229].

At onset of nocardiosis infection, 11 patients (65%) received an imipenem-base induction antimicrobial therapy and 9 (53%) received a TMP-SMX-base consolidation antimicrobial therapy. Details were reported in Table 3. All documented Nocardia were TMP-SMX sensitive.

Immunosuppression and GHVD

In the nocardiosis cohort, GVHD prophylaxis was mostly performed by cyclosporin (CsA) associated to methotrexate for 8 patients (47%). Twelve patients (80%) presented an acute GVHD (aGVHD). All were skin aGVHD with 8 Glückberg's grade 1–2 and 4 more than grade 2. One presented a gut aGVHD (stage 1) and one a liver aGVHD (stage 1). The mean cumulative steroid dose administrated after onset of aGVHD was 25 mg/kg/4weeks [IC95%, 15.5–34.9], compared to 19 mg/kg/4weeks [IC95%, 8.5–28.6] in the control cohort but was not significative ($p = 0.20$) (Table 2).

At nocardiosis diagnosis, 14 patients received immunosuppressive drugs allocated as follow: tacrolimus for 6 (40%), CsA for 4 (27%), high dose prednisone for 2 (13%), JAK2 inhibitor for 1 (6%), JAK2 inhibitor associated to CsA and mycophenolate mofetil, pegylated interferon and lenalidomide for 1. Only one patient had active skin aGVHD (stage 2) and 11 (73%) presented a cGVHD which were mostly moderate (41%) with skin lesions (77%). Thirteen patients received steroid at nocardiosis diagnosis with a prednisone mean dose at 0.3 mg/kg/day [IC95%, 0.19–0.58].

Immune recovery (Figs. 1 and 2A–H)

At nocardiosis diagnosis immune recovery was deficient with a low cellular count for $CD4^+$ T-cells. Subsets including $CD8^+$ T-cells, $CD56^+$ NK cells and $CD19^+$ B-cells counts were in normal ranges. However, immunoglobulin G mean level was low at 4.25 g/L. Means and standard derivations with intervals of confidence were summarized in Table 5. Concerning $CD4^+$ T-cells subpopulations, memory/activated $CD45RA^-/RO^+$ T-cells was preponderant with mean count at 147/μL, naïve $CD4^+$ T-cells production was impaired with naïve $CD4^+CD45RA^+/RO^-$ T-cells (NTE) and $CD4^+CD45RA^+/RO^-/CD31^+$ cells (RTE) means count at 26/μL and 11/μL respectively.

Table 5
Immune reconstitution time points.

	At nocardiosis onset	At day +218 (median time of nocardiosis presentation)		At 24 months after AHCT		Mann–Whitney test
	Nocadiosis cohort	Nocadiosis cohort	Contrôle cohort	Nocadiosis cohort	Contrôle cohort	
Lymphocytes	730/μL [394–1067]	554/μL [356–753]	851/μL [669–1032]	1020/μL [635–1404]	1625/μL [1330–1920]	p = 0.001
CD4+ T-cells	162/μL [80–244]	92/μL [49–134]	153/μL [97–210]	195/μL [102–288]	332/μL [236–429]	p < 0.001
CD4+ CD45RO + T-cells	147/μL [72–223]	83/μL [43–122]	121/μL [72–170]	189/μL [102–277]	256/μL [183–328]	p = 0.02
CD4+ CD45RA + T-cells	26/μL [8–44]	12/μL [6–17]	28/μL [16–40]	17/μL [11–22]	77/μL [46–108]	p < 0.001
CD4+ CD45RA + CD31+ T-cells	11/μL [0–23]	5/μL [2–8]	14/μL [8–21]	14/μL [7–21]	48/μL [23–73]	p < 0.001
CD8+ T-cells	362/μL [125–600]	238/μL [112–363]	403/μL [300–506]	556/μL [297–775]	800/μL [570–1030]	p = 0.001
CD56 + NK cells	160/μL [94–227]	156/μL [97–216]	150/μL [126–173]	141/μL [81–201]	209/μL [153–264]	p = 0.33
CD19 B-cells	112/μL [12–214]	54/μL [8–115]	124/μL [42–206]	131/μL [27–234]	238/μL [137–339]	p = 0.001

Immune reconstitution at time points with cells counts, interval of confidence at 95% and Mann Whitney test.

Assessment of immune recovery over time after AHCT (Fig. 1) highlights a smaller individual variation and an lower cellular count of $CD4^+$ T-cells, $CD45RA^-/RO^+$ T-cells, NTE, RTE, $CD8^+$ T-cells, and $CD19^+$ B-cells in nocardiosis cohort compared to control cohort. CD56 + NK cells distribution was more heterogeneous among both cohorts. Cell counts for the different subsets of immune cells are represented over time in Fig. 2. The case-control comparison of those values were evaluated by Mann–Whitney test (Table 5) and highlighted significant lower cellular count for lymphocytes ($p = 0.001$), $CD4^+$ T-cells ($p < 0.001$), $CD45RA^-/RO^+$ T-cells ($p = 0.02$), NTE ($p < 0.001$) and RTE ($p < 0.001$), $CD8^+$ T-cells ($p = 0.001$) and $CD19^+$ B-cells ($p = 0.001$). $CD56^+$ NK cells cellular count was not different ($p = 0.33$).

At the 7^{th} month after AHCT, the median time of nocardiosis presentation, immune cellular recovery in the nocardiosis cohort was lower comparatively to the control cohort with circulating lymphocytes, $CD4^+$ T-cells, $CD8^+$ T-cells, $CD56^+$ NK cells and $CD19^+$ B-cells means count (Table 5). This relative deficient cellular count was persistent over time like that was presented at 24 months.

Concerning $CD4^+CD45RA^-/RO^+$ T-cells, NTE and RTE mean count, lower $CD4^+$ T-cells in the nocardiosis cohort compared to the control cohort result in a lower cellular count in those three subsets. That was persistent over time after AHCT in the nocardiosis cohort.

Outcomes

Five patients died after nocardiosis but only one death has been directly related to nocardiosis 16 days after diagnosis. The other 4 patients died of relapse of the hematological disease included one arising 32 days after nocardiosis diagnosis with lost of chimerism. No death was related to GVHD or other infection. Donor chimerism has been maintained in those who did not relapse of their hematological disease.

Discussion

Nocardiosis is a rare infection with polymorphic clinical presentation. Characteristics of our rural high-risk population were similar to previous studies [5,31,32] including sex ratio and incidence [33]. Indeed nocardiosis is more frequently diagnosed in dry, windy climates, facilitating aerosolization and dispersal in organism *via* respiratory routes [34,35] and in agricultural workers [7]. The most common infectious site is lung by respiratory tract infestation [6] as shown in our cohort (87%). The common radiological presentation with lungs condensation with abscesses and nodules, and CNS abscesses were similar as already described [2,5,7,8].

Concerning nocardiosis diagnosis over time, we observed a higher incidence over few years. Indeed in a previous epidemiologic evaluation between 2001 and 2012 [36] we shown 5 nocardiosis than we expanded with 5 nocardiosis between 2012 and 2016, and 5 more between 2016 and 2017. Moreover recent use of alternative donor for AHCT (2 haploidenticals, one mismatch 9/10) and sequential conditioning (1 patient) inducing deeper immunosuppression [37] could explain higher incidence over time in our center. Outbreak was already described by other teams [38] but this was not the case in our center. Indeed among the 5 nocardiosis between 2016 and 2017, 4 were documented by PCR with different *Nocardia* species (*N. shimofuensis/higoensis, farcinica, abscessus* and *nova*), 1 was not documented. In another hand, most samples were not routinely kept more than 1 week (48 h for BAL, 5 days for blood cultures) which did not allowed *Nocardia* detection and also contributed to its identification challenge [18]. *Nocardia* PCR permitted more quickly and higher number of *Nocardia* detection. Also six nocardiosis were documented by PCR with specificity reported upon 74% [18]. The issues with that tool arise in case of chronic bronchopulmonary disease, with colonization detection [18]. Widely use of PCR in our center could explain the higher number of nocardiosis. Three PCR were realized on BAL: 1 was associated to a gram-positive bacillus on direct exam impossible to identified, and the 2 others were without any other documentation. None presented a chronic bronchopulmonary disease. Colonization hypothesis for the 2 *Nocardia* isolated in BAL was unlikely considering the severe neurological disseminated presentation for one, and a severe pulmonary infection with radiological compatible presentation for the other.

Time between AHCT and nocardiosis diagnosis was quite different in our study (median time 218 days) compared to previous reports [39]. Usually nocardiosis was presented early (1^{st}-2^{nd} month) and lately (1^{st}-2^{nd} year) after AHCT This particularity may be explained by the proportion of RIC (40%) or alternative AHCT (20%) [37,40]. CsA and high dose steroid (more than 1 mg/kg) use, age at transplantation (mean 49 years-old) and CMV reactivation (26%) were known to altered immune recovery, notably thymic function [41–43]. These characteristics were identical compared to literature for current patients undergoing

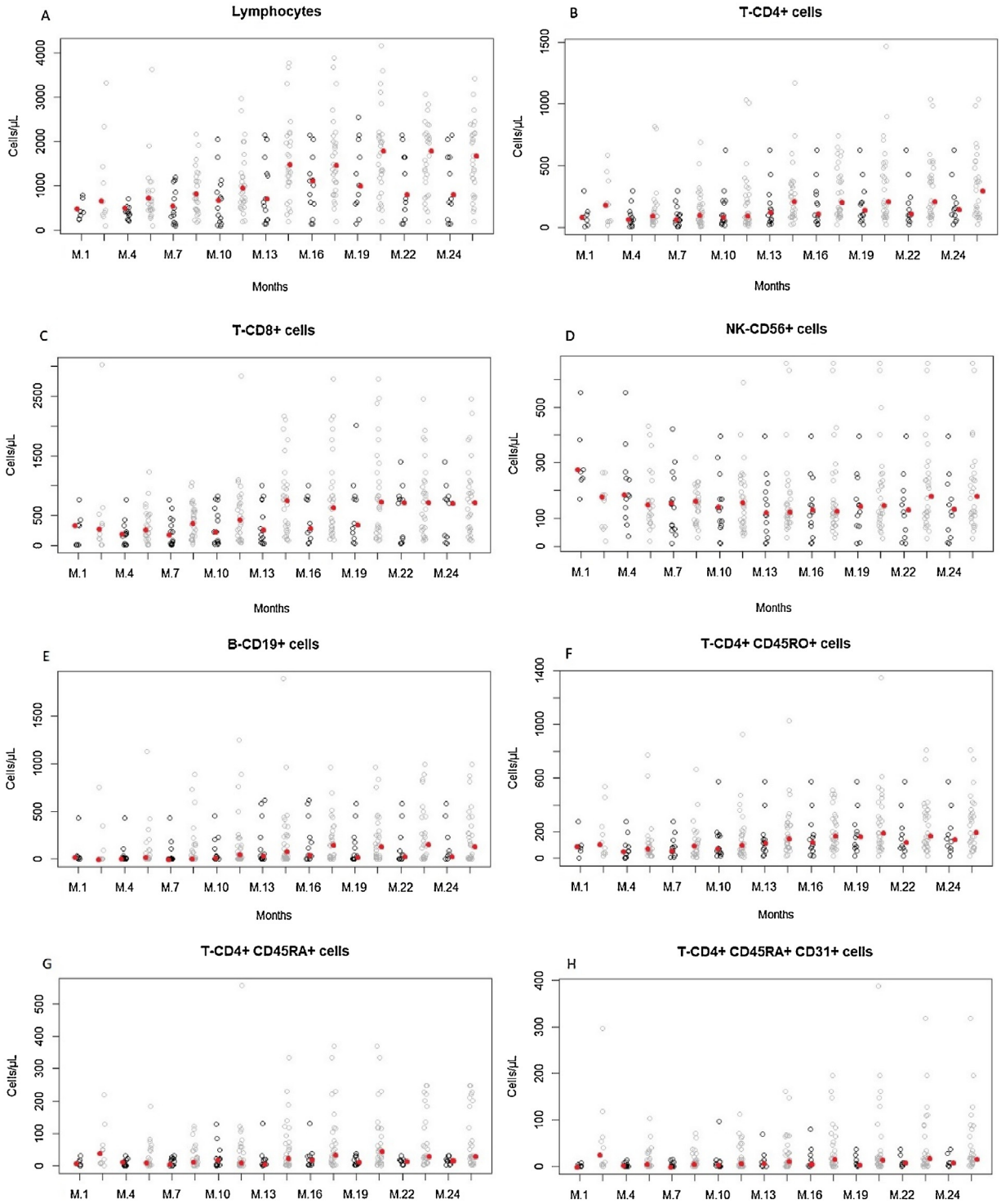

Fig. 1. A–H: Immune recovery over time during 2 years after AHCT in stripchart. Nocardiosis cohort is in back, control cohort is in light grey. Median point is in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to AHCT [44]. Cumulative steroid dose >23 mg/kg/4weeks was also described as an increased risk of infection [45] but we did not permit to observe statistical difference in steroid dose between our 2 cohorts. Half of the patients (8/15) received TMP-SMX prophylaxis at nocardiosis diagnosis. This corroborates that TMP-SMX 3 time a week prophylaxis was not sufficient for nocardiosis prophylaxis as describes in others studies [9,10,16,46,47], but may reduce nocardiosis mortality [48]. However TMP-SMX resistance is rare [49], and poor absorption could be frequent, especially in context of gut GVHD and/or polymedication. Atovaquone is known to be useless [48] for nocardia prophylaxis. Specific recommendations were published by Lebeaux et al. in 2014 about nocardiosis diagnosis and management after AHCT [50] reminding the necessity to use fast molecular diagnosis such PCR and to start a large empirical antimicrobial therapy as soon as Nocardia is suspected (secondary adjusted) that should be maintained over 6–12 months if diagnosis of nocardiosis is confirmed.

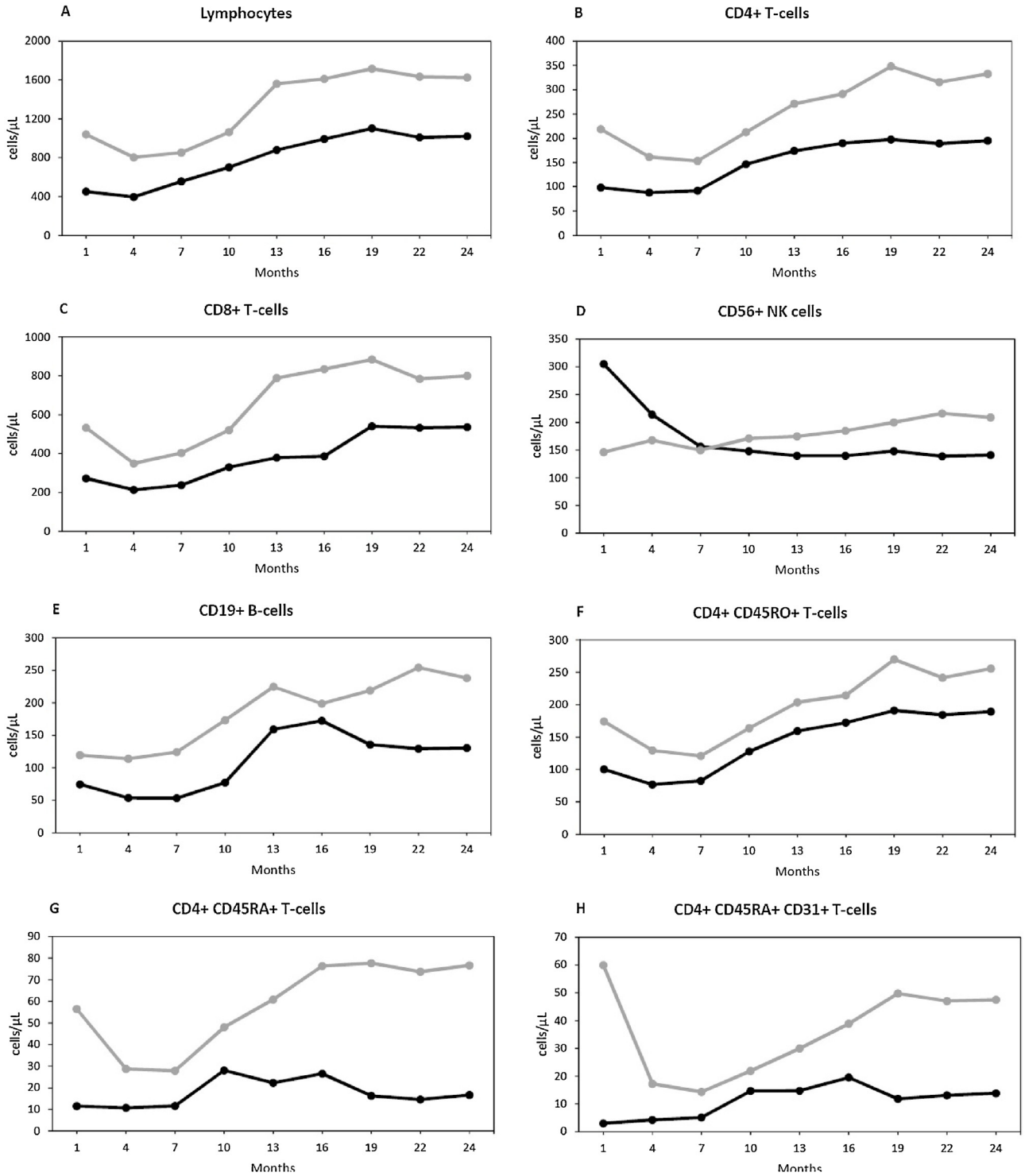

Fig. 2. A–H: Immune recovery over time during 2 years after AHCT with means comparaison. Nocardiosis cohort is in back, control cohort is in grey.

Immune recovery after AHCT is delayed and impaired, frequently described in the literature [37,51]. $CD4^+$ T-cells recovery is delayed to several years after AHCT. Large use of immunosuppressive drugs at nocardiosis diagnosis (88%) delay $CD4^+$ T-cells recovery. Moreover patients with nocardiosis seemed to present a low $CD8^+$ T-cells and $CD19^+$ B-cells recovery, with a significative lower cellular count, that could reflect a higher immunosuppression. Low B-cells count associated to hypogammaglobulinemia may need to consider optimization of immunoglobulin substitution strategy. As already reported, infections may arise despite an acceptable quantitative NK-cells and $CD8^+$ T-cells recovery [51–53], which values were similar to our laboratory benchmarks suggesting a minor implication against *Nocardia*. Immune recovery against *Nocardia* is rather mediated by $CD4^+$ T-cells [54]. Nevertheless in our *Nocardia* cohort normal absolute count $CD4^+$ T-cells was not sufficient to protect against *Nocardia*. $CD4^+$ T-cells recovery after AHCT initially was induced by peripheral clonal expansion of donor memory T cells expressing activation markers ($CD45RA^-/RO^+$). This

initial response is usually followed by central naïve $CD4^+$ T-cells expansion $CD45RA^+/RO^-$ [55–57]. Activated memory T cells recovery in our cohort were shown to be expanded but also failed to prevent nocardiosis. NTE were practically undetectable before and during nocardiosis follow-up. Those descriptions suggest that *Nocardia* did not contribute to delay $CD4^+$ T-cells recovery but was rather a consequence of cellular immune recovery delay.

As same as NTE, RTE count was significantly lower in nocardiosis cohort than control cohort and continued over time. Delay for NTE and RTE repopulation until "normal" level [51,52] was described as a key point that infection risk and mortality risk decreased after AHCT [29,57,58]. Peripheral naïve T-$CD4^+$ cells RTE or not ($CD31^+$ or not) [59] may have equal function but RTE presented a broader polyclonal TCR repertoire compared to non-thymic naïve T-$CD4^+$ cells [60]. TCR repertoire restriction with lack of thymic function may conduct to reduce anti infectious capacity. RTE recovery induces efficient protection against opportunist infection after AHCT [61]. Monitoring efficiency of thymopoiesis by quick tools may allow identifying high-risk patients for opportunistic infection and then personalized.

The analysis of our cohort suggest that the risk of *Nocardia* infection after AHCT is potentially lethal and should be considered with a more intensive laboratory investigation strategy including systematic PCR detection. The assumption of such opportunistic infection is tricky regarding the non specific aspect of its clinical and radiological presentation. Diagnosis should be considered until effective immune cellular recovery, particularly for T-CD4+ cells. We highlight through nocardiosis that the lack of thymopoiesis with low RTE recovery may need a particular attention towards opportunistic infections and prophylaxis with TMP-SMX, even not completely reliable, must be prolonged if necessary. A larger multicentric cohort should be conducted to corroborate those results and to provide more robust recommendations.

Conflict of interest

We have no conflict of interest.

References

[1] Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin Microbiol Rev 2006;19(April (2)):259–82.

[2] Wilson JW. Nocardiosis: updates and clinical overview. Mayo Clin Proc 2012;87(April (4)):403–7.

[3] Beaman BL, Burnside J, Edwards B, Causey W. Nocardial infections in the United States, 1972–1974. J Infect Dis 1976;134(September (3)):286–9.

[4] Beaman BL, Beaman L. Nocardia species: host-parasite relationships. Clin Microbiol Rev 1994;7(April (2)):213.

[5] Minero MV, Marín M, Cercenado E, Rabadán PM, Bouza E, Muñoz P. Nocardiosis at the turn of the century. Medicine (Baltimore) 2009;88(July (4)):250–61.

[6] Chen J, Zhou H, Xu P, Zhang P, Ma S, Zhou J. Clinical and radiographic characteristics of pulmonary nocardiosis: clues to earlier diagnosis. PLoS One 2014;9(March (3))e90724.

[7] Ambrosioni J, Lew D, Garbino J. Nocardiosis: updated clinical review and experience at a tertiary center. Infection 2010;38(April (2)):89–97.

[8] Orlowski HLP, McWilliams S, Mellnick VM, Bhalla S, Lubner MG, Pickhardt PJ, et al. Imaging spectrum of invasive fungal and fungal-like infections. Radiogr Rev Publ Radiol Soc N Am Inc 2017;37(August (4)):1119–34.

[9] van Burik J-A, Hackman RC, Nadeem SQ, Hiemenz JW, White MH, Flowers MED, et al. Nocardiosis after bone marrow transplantation: a retrospective study. Clin Infect Dis 1997;24(June (6)):1154–60.

[10] Coussement J, Lebeaux D, Rouzaud C. Lortholary *O. nocardia* infections in solid organ and hematopoietic stem cell transplant recipients. Curr Opin Infect Dis 2017;30(December (6)):545–51.

[11] Atsuta Y, Hirakawa A, Nakasone H, Kurosawa S, Oshima K, Sakai R, et al. Late mortality and causes of death among long-term survivors after allogeneic stem cell transplantation. Biol Blood Marrow Transplant 2016;22(September (9):1702–9.

[12] D'Souza A, Fretham C. Current uses and outcomes of hematopoietic cell transplantation (HCT): CIBMTR summary slides. Available at. 2018. https://www.cibmtr.org.

[13] Jacobson CA, Turki AT, McDonough SM, Stevenson KE, Kim HT, Kao G, et al. Immune reconstitution after double umbilical cord blood stem cell transplantation: comparison with unrelated peripheral blood stem cell transplantation. Biol Blood Marrow Transplant 2012;18(April (4)):565–74.

[14] Fayard A, Daguenet E, Blaise D, Chevallier P, Labussière H, Berceanu A, et al. Evaluation of infectious complications after haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide following reduced-intensity and myeloablative conditioning: a study on behalf of the Francophone Society of Stem Cell Transplantation and Cellular Therapy (SFGM-TC). Bone Marrow Transplant 2019;1(February).

[15] Miller HK, Braun TM, Stillwell T, Harris AC, Choi S, Connelly J, et al. Infectious risk after allogeneic hematopoietic cell transplantation complicated by acute graft-versus-host disease. Biol Blood Marrow Transplant 2017;23(March (3)):522–8.

[16] Shannon K, Pasikhova Y, Ibekweh Q, Ludlow S, Baluch A. Nocardiosis following hematopoietic stem cell transplantation. Transpl Infect Dis Off J Transplant Soc 2016;18(April (2)):169–75.

[17] De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European organization for research and treatment of cancer/invasive fungal infections cooperative group and the national institute of allergy and infectious diseases mycoses study group (EORTC/MSG) consensus group. Clin Infect Dis Off Publ Infect Dis Soc Am. 2008;46(June (12)):1813–21.

[18] Rouzaud C, Rodriguez-Nava V, Catherinot E, Méchaï F, Bergeron E, Farfour E, et al. Clinical assessment of a *Nocardia* spp. polymerase chain reaction (PCR)-based assay for the diagnosis of nocardiosis. J Clin Microbiol 2018(March) JCM.00002-18.

[19] Sorror ML, Logan BR, Zhu X, Rizzo JD, Cooke KR, McCarthy PL, et al. Prospective validation of the predictive power of the hematopoietic cell transplantation comorbidity index: a center for international blood and marrow transplant research study. Biol Blood Marrow Transplant 2015;21(August (8)):1479–87.

[20] Averbuch D, Orasch C, Cordonnier C, Livermore DM, Mikulska M, Viscoli C, et al. European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European conference on Infections in Leukemia. Haematologica 2013;98 (December (12)):1826–35.

[21] Styczynski J, Reusser P, Einsele H, de la Camara R, Cordonnier C, Ward KN, et al. Management of HSV, VZV and EBV infections in patients with hematological malignancies and after SCT: guidelines from the second European conference on infections in Leukemia. Bone Marrow Transplant 2009;43(May (10)): 757–70.

[22] Rizzo JD, Wingard JR, Tichelli A, Lee SJ, Van Lint MT, Burns LJ, et al. Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation: joint recommendations of the European Group for Blood and Marrow Transplantation, Center for International Blood and Marrow Transplant Research and the American Society for Blood and Marrow Transplantation (EBMT/CIBMTR/ASBMT). Bone Marrow Transplant 2006;37(February (3)):249–61.

[23] Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect 2018;24(May): e1–e38.

[24] Maertens J, Cesaro S, Maschmeyer G, Einsele H, Donnelly JP, Alanio A, et al. ECIL guidelines for preventing *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients. J Antimicrob Chemother 2016;71(Sep (9)):2397–404.

[25] Ljungman P, Engelhard D, de la Cámara R, Einsele H, Locasciulli A, Martino R, et al. Vaccination of stem cell transplant recipients: recommendations of the infectious diseases working party of the EBMT. Bone Marrow Transplant 2005;35(April (8)):737–46.

[26] Sullivan KM, Dykewicz CA, Longworth DL, Boeckh M, Baden LR, Rubin RH, et al. Preventing opportunistic infections after hematopoietic stem cell transplantation: the Centers for Disease Control and Prevention, Infectious Diseases Society of America, and American Society for Blood and Marrow Transplantation Practice Guidelines and Beyond. ASH Educ Program Book 2001;2001(January (1)):392–421.

[27] Millon L, Larosa F, Lepiller Q, Legrand F, Rocchi S, Daguindau E, et al. Quantitative polymerase chain reaction detection of circulating DNA in serum for early diagnosis of mucormycosis in immunocompromised patients. Clin Infect Dis 2013;56(May (10)):e95–e101.

[28] Toussirot É, Saas P, Deschamps M, Pouthier F, Perrot L, Perruche S, et al. Increased production of soluble CTLA-4 in patients with spondylarthropathies correlates with disease activity. Arthritis Res Ther 2009;11(July):R101.

[29] Larosa F, Maddens S, Legrand F, Pouthier F, Ferrand C, Saas P, et al. Early immune reconstitution and efficient graft vs tumor effect after unrelated partially matched double cord blood transplantation in refractory 8p11 syndrome. Bone Marrow Transplant 2011;46(April (4)):622–4.

[30] Thiede C, Florek M, Bornhäuser M, Ritter M, Mohr B, Brendel C, et al. Rapid quantification of mixed chimerism using multiplex amplification of short tandem repeat markers and fluorescence detection. Bone Marrow Transplant 1999;23(May (10)):1055–60.

[31] Tremblay J, Thibert L, Alarie I, Valiquette L, Pépin J. Nocardiosis in Quebec, Canada, 1988–2008. Clin Microbiol Infect 2011;17(May (5)):690–6.

[32] van de Sande WWJ. Global burden of human mycetoma: a systematic review and meta-analysis. PLoS Negl Trop Dis 2013;7(November (11))e2550.

[33] Cattaneo C, Antoniazzi F, Caira M, Castagnola C, Delia M, Tumbarello M, et al. Nocardia spp infections among hematological patients: results of a retrospective multicenter study. Int J Infect Dis 2013;17(8):e610–4.
[34] Saubolle MA, Sussland D. Nocardiosis review of clinical and laboratory experience. J Clin Microbiol 2003;41(January (10)):4497–501.
[35] Clark NM, Reid GE, the AST Infectious Diseases Community of Practice. Nocardia infections in solid organ transplantation. Am J Transplant 2013;13 (March (s4)):83–92.
[36] Mansi L, Daguindau E, Saas P, Pouthier F, Ferrand C, Dormoy A, et al. Diagnosis and management of nocardiosis after bone marrow stem cell transplantation in adults: lack of lymphocyte recovery as a major contributing factor. Pathol Biol 2014;62(3):156–61.
[37] Servais S, Lengline E, Porcher R, Carmagnat M, de Latour RP, Robin M, et al. Long-term immune reconstitution and infection burden after mismatched hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2014;20(April (4)):507–17.
[38] Kachi S, Okazaki M, Takeda H, Igarashi H, Kobayashi O, Watanabe H, et al. Outbreak of *Nocardia farcinica* infection with the same pattern in randomly amplified polymorphic DNA analysis. J Hosp Infect 2006;62(April (4)): 502–6.
[39] Chouciño C, Goodman SA, Greer JP, Stein RS, Wolff SN, Dummer JS. Nocardial infections in bone marrow transplant recipients. Clin Infect Dis 1996;23 (November (5)):1012–9.
[40] Fukuda T, Boeckh M, Carter RA, Sandmaier BM, Maris MB, Maloney DG, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood 2003;102(August (3)):827–33.
[41] Arduino RC, Johnson PC, Miranda braham G. Nocardiosis in renal transplant recipients undergoing immunosuppression with cyclosporine. Clin Infect Dis 1993;16(April (4)):505–12.
[42] Martínez Tomás R, Menéndez Villanueva R, Reyes Calzada S, Santos Durantez M, Vallés Tarazona JM, Modesto Alapont M, et al. Pulmonary nocardiosis: risk factors and outcomes. Respirology 2007;12(May (3)):394–400.
[43] Nishida R, Mori Y, Iwasaki H, Tokuyama T, Kamezaki K, Nagasaki Y, et al. Pulmonary nocardiosis developed in a hematopoietic stem cell transplant recipient with bronchiolitis obliterans. Intern Med 2010;49(14):1441–4.
[44] Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med 2010;363(November (22)):2091–101.
[45] Matsumura-Kimoto Y, Inamoto Y, Tajima K, Kawajiri A, Tanaka T, Hirakawa T, et al. Association of cumulative steroid dose with risk of infection after treatment for severe acute graft-versus-Host disease. Biol Blood Marrow Transplant 2016;22(June (6)):1102–7.
[46] Peleg AY, Husain S, Qureshi ZA, Silveira FP, Sarumi M, Shutt KA, et al. Risk factors, clinical characteristics, and outcome of Nocardia infection in organ transplant recipients: a matched case-control study. Clin Infect Dis 2007;44 (May (10)):1307–14.
[47] Hemmersbach-Miller M, Stout JE, Woodworth MH, Cox GM, Saullo JL. Nocardia infections in the transplanted host. Transpl Infect Dis 2018;0(0)e12902.
[48] Molina A, Winston DJ, Pan D, Schiller GJ. Increased incidence of nocardial infections in an era of atovaquone prophylaxis in allogeneic hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant 2018;24(August (8)):1715–20.
[49] Schlaberg R, Fisher MA, Hanson KE. Susceptibility profiles of *Nocardia* isolates based on current taxonomy. Antimicrob Agents Chemother 2014;58(January (2)):795–800.
[50] Lebeaux D, Morelon E, Suarez F, Lanternier F, Scemla A, Frange P, et al. Nocardiosis in transplant recipients. Eur J Clin Microbiol Infect Dis 2014;33 (May (5)):689–702.
[51] Geddes M, Storek J. Immune reconstitution following hematopoietic stem-cell transplantation. Best Pract Res Clin Haematol 2007;20(June (2)):329–48.
[52] Storek J, Joseph A, Espino G, Dawson MA, Douek DC, Sullivan KM, et al. Immunity of patients surviving 20 to 30 years after allogeneic or syngeneic bone marrow transplantation. Blood 2001;98(December (13)):3505–12.
[53] Weinberg K, Blazar BR, Wagner JE, Agura E, Hill BJ, Smogorzewska M, et al. Factors affecting thymic function after allogeneic hematopoietic stem cell transplantation. Blood 2001;97(March (5)):1458–66.
[54] Deem RL, Doughty FA, Beaman BL. Immunologically specific direct T lymphocyte-mediated killing of *Nocardia asteroides*. J Immunol 1983;130 (May (5)):2401–6.
[55] Bengtsson M, Tötterman TH, Smedmyr B, Festin R, Oberg G, Simonsson B. Regeneration of functional and activated NK and T sub-subset cells in the marrow and blood after autologous bone marrow transplantation: a prospective phenotypic study with 2/3-color FACS analysis. Leukemia 1989;3 (January (1)):68–75.
[56] Sugita K, Soiffer RJ, Murray C, Schlossman SF, Ritz J, Morimoto C. The phenotype and reconstitution of immunoregulatory T cell subsets after T cell-depleted allogeneic and autologous bone marrow transplantation. Transplantation 1994;57(May (10)):1465–73.
[57] Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, et al. Changes in thymic function with age and during the treatment of HIV infection. Nature 1998;396(December (6712)):690–5.
[58] Storek J, Witherspoon RP, Storb R. T cell reconstitution after bone marrow transplantation into adult patients does not resemble T cell development in early life. Bone Marrow Transplant 1995;16(September (3)):413–25.
[59] Kimmig S, Przybylski GK, Schmidt CA, Laurisch K, Möwes B, Radbruch A, et al. Two subsets of Naive T helper cells with distinct T cell receptor excision circle content in human adult peripheral blood. J Exp Med 2002;195(March (6)):789–94.
[60] Kohler S, Thiel A. Life after the thymus: CD31+ and CD31− human naive CD4+ T-cell subsets. Blood 2009;113(January (4)):769–74.
[61] Thiel A, Alexander T, Schmidt CA, Przybylski GK, Kimmig S, Kohler S, et al. Direct assessment of thymic reactivation after autologous stem cell transplantation. Acta Haematol 2008;119(1):22–7.