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Altered thymic CD4⁺ T-cell recovery after allogeneic hematopoietic stem cell transplantation is critical for Nocardiosis.

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Running title: Nocardiosis and thymic recovery

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1 **Altered thymic CD4⁺ T-cell recovery after allogeneic hematopoietic stem cell**
2 **transplantation is critical for Nocardiosis.**

3
4 **Abstract**

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

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

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

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

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33
34
35 **Key words:** *Nocardia*, opportunistic infection, allogeneic hematopoietic stem cell transplant,
36 immune recovery, thymic CD4⁺ T-cell.
37

38 **Introduction**

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

45 Nocardiosis is the name of *Nocardia spp* infection, and could affect immunocompromised
46 human hosts [3]. This opportunistic pathogen affects particularly patients exhibiting an altered
47 cell-mediated immunity [4]. Solid-organ and hematopoietic stem cell transplant recipients
48 concerned one third of nocardiosis [2,5]. The clinical presentation is heterogeneous, non-
49 specific and depends on infectious sites [4]. The most common infectious site is lung by
50 respiratory tract infestation [6], nevertheless other localizations with disseminated presentation
51 could be presented in blood, central nervous system (CNS) or lymph nodes [7]. The common
52 radiological presentation does not have pathognomonic features as it shows lungs
53 condensation with abscesses and nodules. CNS dissemination is also usually described with
54 parenchymal abscesses [2,5,7,8]. As rare and late after allogeneic hematopoietic cell
55 transplantation (AHCT), nocardiosis is potentially lethal with a survival rate reaching 84% (in
56 retrospective studies) [9]. Gathering cohorts of immunosuppressed patients, incidence is
57 estimated from 0.3 to 3.6% [2,9]. This incidence is likely under-estimated [5] as result of
58 *Nocardia* identification challenge [10].

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

67

68 **Patients and methods**

69 We described nocardiosis characteristics, management and outcome after AHCT in a
70 hematopoietic cell transplant recipients' cohort treated at the University Hospital of Besançon.
71 We analyzed immune reconstitution through circulating T-cells populations that were
72 phenotyped before, at onset and after nocardiosis. The different circulating lymphoid
73 populations available for analysis included: NK cells, CD8⁺ T-cells, CD4⁺ T-cells and CD19⁺ B-

74 cells. Our regular panel assessing immune reconstitution focused more precisely on naive
75 thymic emigrant CD4⁺ T-cells (NTE identified as CD45RA⁺/RO⁻) and recent thymic emigrants
76 CD4⁺ T-cells (RTE identified as CD45RA⁺/RO⁻/CD31⁺) to appreciate thymic output impact
77 after transplantation.

78 **Patients**

79 Patients were selected from the local clinical data base between May 2001 and December
80 2017. We included all nocardiosis cases defined as “proven”, “probable” or “possible” based
81 on the similar invasive fungal infection (IFI) classification [17]. We defined “possible”
82 nocardiosis by the presence of clinical and radiological features (nodule, condensation,
83 abscess), with or without dissemination [8], no alternative diagnosis and improvement after
84 anti-*Nocardia* treatment in an immunocompromised host. “Probable” nocardia was defined by
85 identification of non-direct bacteriological criteria (direct exam, pathology, positive *Nocardia*
86 PCR), and “proven” by positive *Nocardia* culture confirmation, as described recently [18] (table
87 1). Pathology arguments to bacterial infection or gram-positive bacillus were considered as
88 non-direct bacteriological criteria.

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

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

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

102 **Statistic methods**

103 Statistical analyses and graphs were performed with Rstudio® Version 1.1.463. Continuous
104 variables were summarized with means and standard deviation with interval of confidence at
105 95%. All observations over time were represented on stripcharts with identification of median
106 value. Immune recovery was evaluated at least every three months during the first 2 years
107 (more frequently for frail patients with GVHD or recurrent infection) and compared with the

108 control population. Patients were censored at death time. Means of T-cell subset count were
109 compared by Mann-Whitney test.

110 **AHCT management**

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

114 During the first month after AHCT, all patients were hospitalized in High Efficiency Particulate
115 Air laminar flow chamber until neutrophilic recovery. An intravenous antibiotic therapy was
116 administered empirically or adjusted to patient's environment, in case of fever [20]. Bacterial,
117 viral and fungal prophylaxis were administered as already described: long-term valaciclovir
118 [21], fluconazole or posaconazole during 6 months or more in case of GVHD [22,23],
119 trimethoprim-sulfamethoxazole (TMP-SMX 800mg/160mg) three time a week, or in case of
120 adverse event atovaquone or aerosol of pentamidine [24]. Intravenous polyvalent
121 immunoglobulins were administrated (400mg/kg) in case of immunoglobulin G <3g/L. Vaccines
122 were administered according to EBMT schedules [25] and adapted to the immune recovery.

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

135 **Specific microbiological detection of *Nocardia***

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

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

143 **Immune recovery assessment**

144 Lymphocytes counts were evaluated by XN-10 system automat (Sysmex, Japan). Absolute
145 lymphocytes, CD4⁺ T-cells, CD8⁺ T-cells, CD56⁺ NK cells and CD19⁺ B-cells counts were
146 determined by flow cytometry by TetraCXP1 method and fluorescence evaluation by FC500
147 cytometer (Beckman Coulter, France) [28]. Naïve CD4⁺ T-cells population was characterized
148 by CD45RA⁺/CD45RO⁻/CD4⁺ labeling in immunofluorescence. CD31⁺ expression was
149 evaluated in order to identify RTE lymphocytes and subsequent thymic lymphocyte maturation
150 [29]. Our laboratory benchmark for general healthy population was 493-1666 CD4⁺ T-cells/μL,
151 224-1112 CD8⁺ T-cells/μL, 75-520 CD19⁺ B-cells/μL and 73-654 CD56⁺ NK cells/μL.
152 Quantitative immunoglobulin evaluation was also performed to quantitatively assess the humoral
153 immune recovery.

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

158

159 **Results**

160 **Population**

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

163 In the nocardiosis cohort one patient received 2 AHCT, acute myeloid leukemia (AML) was the
164 most frequent hematological disease (61%), 10 patients (63%) were in complete response
165 (CR) at the transplantation time, 2 in partial response, 3 in progressive disease. Males were
166 preponderant (sex ratio 4:1) (table 2). Myeloablative conditioning regimens (MAC) were used
167 for 9 patients (60%) and the 6 others conditioning regimens (40%) were reduce intensity
168 conditionings (RIC). Ten patients (77%) received anti-thymoglobulin serum for GVHD
169 prophylaxis during conditioning regimen (2.5 mg/kg for 1 patient, 5 mg/kg for 8 and 7.5 mg/kg
170 for one). Median CD34⁺ infused cells count was 2.69 x 10⁶/kg [IC 95%, 1.85-3.57].
171 Characteristics were similar to control cohort as report in table 2.

172 **Nocardiosis presentation**

173 Six nocardiosis occurred in patient receiving AHCT in spring whereas others were equally
174 presented in summer, autumn and winter. Median duration time between AHCT and
175 nocardiosis diagnosis was 218 days [IC95%, 118-317]. On the 15 nocardiosis, 7 (47%) were
176 localized then 8 (53%) were disseminated (at least 2 infectious sites). Thirteen (87%)
177 presented a pulmonary involvement, 8 (53%) were neurological nocardiosis with central

178 nervous system (CNS) involvement with 4 (24%) ocular involvement, 2 (12%) were skin
179 nocardiosis and 1 was a bacteriema (table 3 - 4). Main radiological presentation was
180 abscesses (11/15, 73%), with lungs nodules (7/13, 53%) and condensation (9/13, 69%) half
181 time associated to abscesses (5/13, 38%), and CNS abscesses (8/8). As previously defined,
182 5 nocardiosis cases were proved, 6 were probable, 4 were possible (table 4). Ten (66%) were
183 documented by PCR, 3 (20%) by cyto-histopathology, 5 (33%) by late culture with 1 (6%) on
184 blood culture. Five patients had nocardia detection through at least 2 different methods. Four
185 patients had missing documentation : 3 without possible laboratory *Nocardia* detection on
186 samples and one which the sample was lost. Nevertheless they were highly suspected based
187 on clinical and radiology presentation and because of the negative regular microbiological
188 investigation regard and the favorable evolution under specific anti-*Nocardia* treatment.

189 Two patients presented a nocardiosis recurrence : one after a second AHCT 3 years later and
190 the other one 2 months after anti-microbial prophylaxis ending. For this patient, the secondary
191 prophylaxis had been maintained Eight patients received TMP-SMX prophylaxis at
192 nocardiosis diagnosis and relapse. Among them, 3 had gut GVHD. Four patients presented
193 concomitant CMV reactivation and 4 other patients presented co-infection (2 *Mucormycosis*
194 and 2 *Pneumocystis*). One of the patients with pneumocystosis did not followed the prescribed
195 TMP-SMX prophylaxis. Median duration time of antimicrobial treatment was 180 days [IC95%,
196 130-229].

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

200 **Immunosuppression and GHVD**

201 In the nocardiosis cohort, GVHD prophylaxis was mostly performed by cyclosporin (CsA)
202 associated to methotrexate for 8 patients (47%). Twelve patients (80%) presented an acute
203 GVHD (aGVHD). All were skin aGVHD with 8 Glückberg's grade 1-2 and 4 more than grade
204 2. One presented a gut aGVHD (stage 1) and one a liver aGVHD (stage 1). The mean
205 cumulative steroid dose administrated after onset of aGVHD was 25mg/kg/4weeks [IC95%,
206 15.5-34.9], compared to 19mg/kg/4weeks [IC95%, 8.5-28.6] in the control cohort but was not
207 significant ($p=0.20$) (table 2).

208 At nocardiosis diagnosis, 14 patients received immunosuppressive drugs allocated as follow :
209 tacrolimus for 6 (40%), CsA for 4 (27%) , high dose prednisone for 2 (13%), JAK2 inhibitor for
210 1 (6%) , JAK2 inhibitor associated to CsA and mycophenolate mofetil, pegylated interferon and
211 lenalidomide for 1. Only one patient had active skin aGVHD (stage 2) and 11 (73%) presented
212 a cGVHD which were mostly moderate (41%) with skin lesions (77%). Thirteen patients

213 received steroid at nocardiosis diagnosis with a prednisone mean dose at 0.3 mg/kg/day
214 [IC95%, 0.19-0.58].

215 **Immune recovery** (figures 1 and 2 A-H)

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

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

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

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

241 **Outcomes**

242 Five patients died after nocardiosis but only one death has been directly related to nocardiosis
243 16 days after diagnosis. The other 4 patients died of relapse of the hematological disease
244 included one arising 32 days after nocardiosis diagnosis with lost of chimerism. No death was
245 related to GVHD or other infection. Donor chimerism has been maintained in those who did
246 not relapse of their hematological disease.

247

248 Discussion

249 Nocardiosis is a rare infection with polymorphic clinical presentation. Characteristics of our
250 rural high-risk population were similar to previous studies[5,31,32] including sex ratio and
251 incidence [33]. Indeed nocardiosis is more frequently diagnosed in dry, windy climates,
252 facilitating aerosolization and dispersal in organism *via* respiratory routes [34,35] and in
253 agricultural workers [7]. The most common infectious site is lung by respiratory tract infestation
254 [6] as shown in our cohort (87%). The common radiological presentation with lungs
255 condensation with abscesses and nodules, and CNS abscesses were similar as already
256 described [2,5,7,8].

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

278 Time between AHCT and nocardiosis diagnosis was quite different in our study (median time
279 218 days) compared to previous reports [39]. Usually nocardiosis was presented early (1st-2nd
280 month) and lately (1st-2nd year) after AHCT This particularity may be explained by the
281 proportion of RIC (40%) or alternative AHCT (20%) [37,40]. CsA and high dose steroid (more
282 than 1mg/kg) use, age at transplantation (mean 49 years-old) and CMV reactivation (26%)
283 were known to altered immune recovery, notably thymic function [41–43]. These

284 characteristics were identical compared to literature for current patients undergoing to AHCT
285 [44]. Cumulative steroid dose >23mg/kg/4weeks was also described as an increased risk of
286 infection [45] but we did not permit to observe statistical difference in steroid dose between our
287 2 cohorts. Half of the patients (8/15) received TMP-SMX prophylaxis at nocardiosis diagnosis.
288 This corroborates that TMP-SMX 3 time a week prophylaxis was not sufficient for nocardiosis
289 prophylaxis as describes in others studies [9,10,16,46,47], but may reduce nocardiosis
290 mortality [48]. However TMP-SMX resistance is rare [49], and poor absorption could be
291 frequent, especially in context of gut GVHD and/or polymedication. Atovaquone is known to
292 be useless [48] for nocardia prophylaxis. Specific recommendations were published by
293 *Lebeaux et al.* in 2014 about nocardiosis diagnosis and management after AHCT [50]
294 reminding the necessity to use fast molecular diagnosis such PCR and to start a large empirical
295 antimicrobial therapy as soon as *Nocardia* is suspected (secondary adjusted) that should be
296 maintained over 6 to 12 months if diagnosis of nocardiosis is confirmed.

297 Immune recovery after AHCT is delayed and impaired, frequently described in the literature
298 [37,51]. CD4⁺ T-cells recovery is delayed to several years after AHCT. Large use of
299 immunosuppressive drugs at nocardiosis diagnosis (88%) delay CD4⁺ T-cells recovery.
300 Moreover patients with nocardiosis seemed to present a low CD8⁺ T-cells and CD19⁺ B-cells
301 recovery, with a significative lower cellular count, that could reflect a higher
302 immunosuppression. Low B-cells count associated to hypogammaglobulinemia may need to
303 consider optimization of immunoglobulin substitution strategy. As already reported, infections
304 may arise despite an acceptable quantitative NK-cells and CD8⁺ T-cells recovery [51–53],
305 which values were similar to our laboratory benchmarks suggesting a minor implication against
306 *Nocardia*. Immune recovery against *Nocardia* is rather mediated by CD4⁺ T-cells [54].
307 Nevertheless in our *Nocardia* cohort normal absolute count CD4⁺ T-cells was not sufficient to
308 protect against *Nocardia*. CD4⁺ T-cells recovery after AHCT initially was induced by peripheral
309 clonal expansion of donor memory T cells expressing activation markers (CD45RA⁺/RO⁺). This
310 initial response is usually followed by central naïve CD4⁺ T-cells expansion CD45RA⁺/RO⁻ [55–
311 57]. Activated memory T cells recovery in our cohort were shown to be expanded but also
312 failed to prevent nocardiosis. NTE were practically undetectable before and during nocardiosis
313 follow-up. Those descriptions suggest that *Nocardia* did not contribute to delay CD4⁺ T-cells
314 recovery but was rather a consequence of cellular immune recovery delay.

315 As same as NTE, RTE count was significantly lower in nocardiosis cohort than control cohort
316 and continued over time. Delay for NTE and RTE repopulation until “normal” level [51,52] was
317 described as a key point that infection risk and mortality risk decreased after AHCT [29,57,58].
318 Peripheral naïve T-CD4⁺ cells RTE or not (CD31⁺ or not) [59] may have equal function but RTE
319 presented a broader polyclonal TCR repertoire compared to non-thymic naïve T-CD4⁺ cells

320 [60]. TCR repertoire restriction with lack of thymic function may conduct to reduce anti
321 infectious capacity. RTE recovery induces efficient protection against opportunist infection
322 after AHCT [61]. Monitoring efficiency of thymopoiesis by quick tools may allow identifying
323 high-risk patients for opportunistic infection and then personalized

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

333

334

335 **Conflict of interest**

336 We have no Conflict of Interest.

337

338

339

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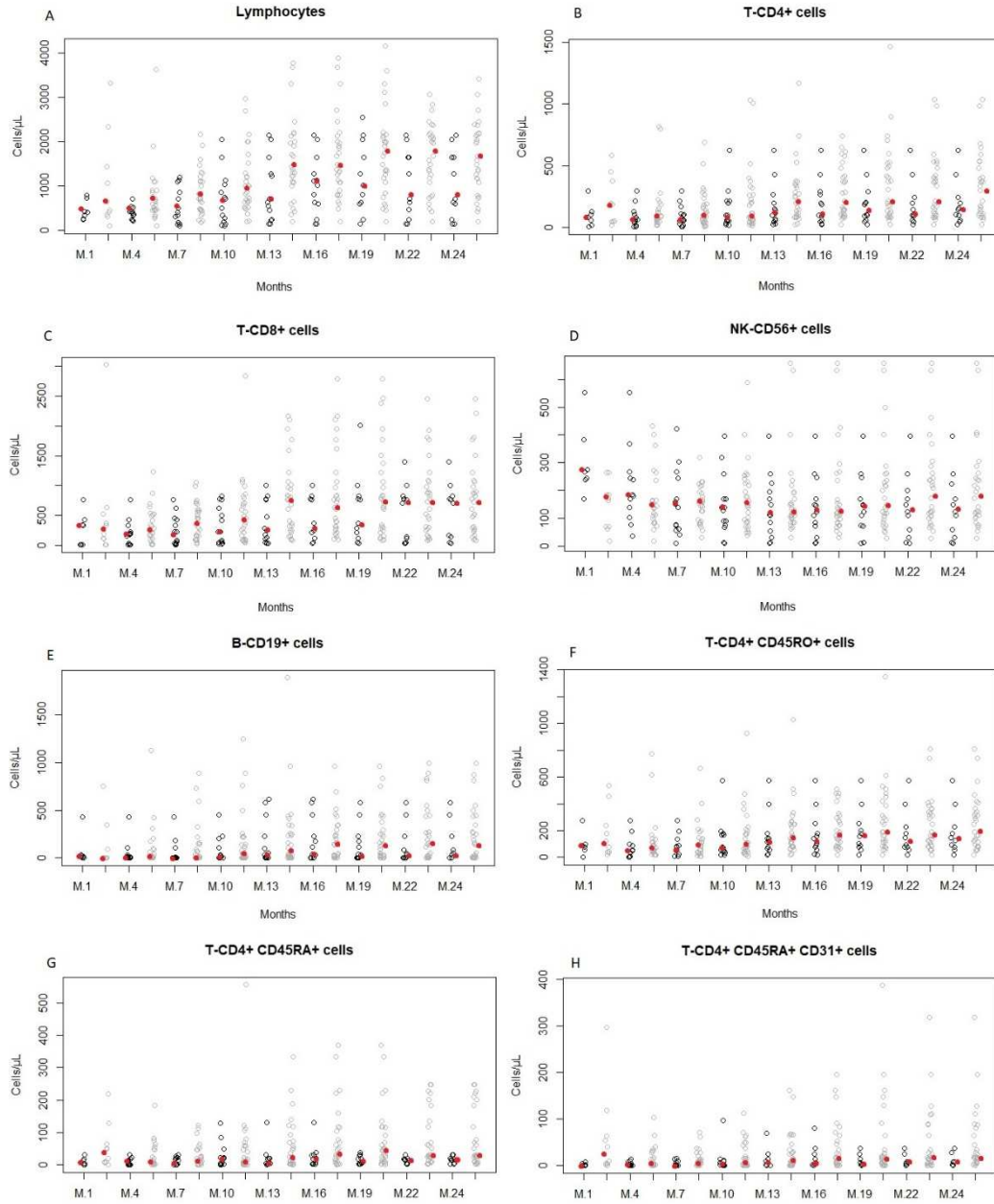


Fig 1 A-H : Immune recovery over time during 2 years after AHCT in stripchart. Nocardiosis cohort is in back, control cohort is in light grey. Median point is in red.

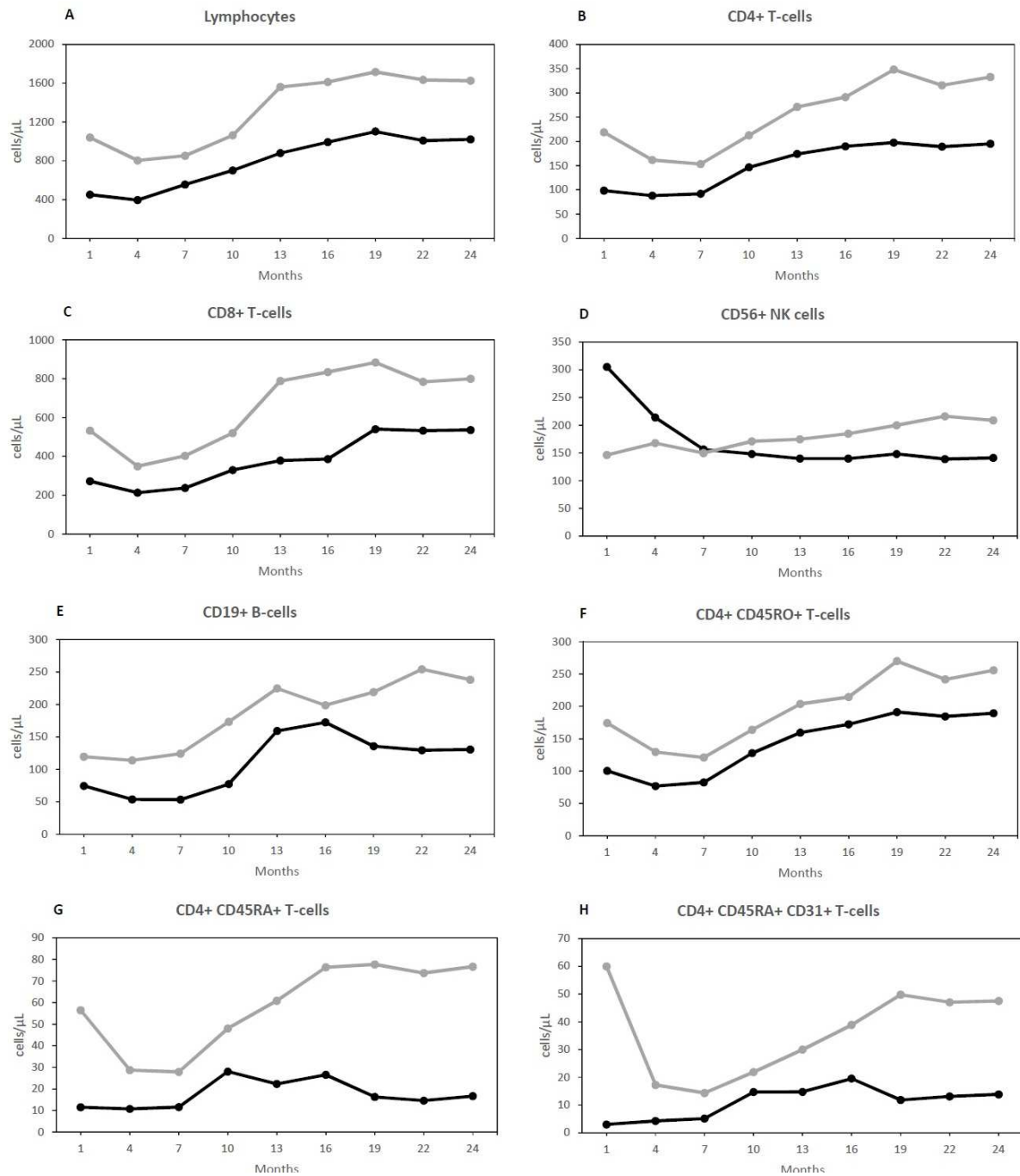


Fig 2 A-H : Immune recovery over time during 2 years after AHCT with means comparison. Nocardiosis cohort is in back, control cohort is in grey.

(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

(Table 2) Transplant characteristics		
	<u>Nocardiosis cohort</u>	<u>Control cohort</u>
Mean age (years) (p=0.11)	49 [43-55]	43 [37-49]
<u>Sex ratio</u>	4:1	4:1
<u>Hematological disease</u>		
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%)
<u>Conditioning</u>		
Myeloablative conditioning	9 (60%)	18 (60%)
Reduce intensity conditioning	6 (40%)	12 (40%)
<u>HSCT type</u>		
Bone marrow	8 (54%)	16 (54%)
Peripheral blood stem cells	7 (46%)	14 (46%)
<u>Donor type</u>		
Sibling	8 (54%)	16 (54%)
Unrelated donor	4 (27%)	10 (33%)
9/10	1 (6%)	-
Haplo identical	2 (13%)	4 (13%)
<u>CMV donor/recipient status</u>		
+ / +	4 (27%)	11 (37%)
+ / -	3 (20%)	2 (6%)
- / +	5 (33%)	9 (30%)
- / -	3 (20%)	8 (27%)
<u>Graft-versus-host disease</u>		
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%)
<u>Steroid cumulative dose</u>		
Mean during 1 st 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)			
<u>Prophylaxis (TMP-SMX)</u>	8 (53%)	<u>Antibiotic treatment (n=17)</u>	
		Induction	
<u>Infection status</u>		Ceftriaxone	1 (6%)
Localized	7 (47%)	Ceftriaxone + Linezolid	1 (6%)
Disseminated	8 (53%)	IMP	5 (29%)
		IMP + AMK	3 (18%)
<u>Infection site</u>		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	
<u>Documentation</u>		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%)
<u>Co-infection</u>		Ceftriaxone	2 (12%)
CMV	4 (26%)	MERO + Linezolid	1 (6%)
Pneumocystis	2 (13%)	Minocycline + Ciprofloxacin	1 (6%)
Mucormycosis	2 (13%)	Dead before	2 (12%)
<u>Mean steroid dose at diagnosis</u>	0.30mg/kg/day [0.19-0.58]		
IMP imipenem, MERO meropenem, AMK amikacin, TMP-SMX trimethoprim-sulfamethoxazole, CNS Central Nervous System			

(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	+	+	+	<i>N. cyriacigeorgica</i>	Proved
#2	None	Skin	Smooth tissue CT scan : abscess	Skin biopsy	-	+	+	<i>N. farcinica</i>	Proved
#3	TMP-SMX	CNS, Oc, Lung	Cerebral MRI : multiple abscesses + Chest CT scan : lungs nodules	Lung biopsy	indirect	+	+	<i>N. farcinica</i>	Proved
#4	TMP-SMX	CNS, Oc, Lung	Cerebral CT scan : multiple abscesses + Chest CT scan : lung nodule	BAL	-	not made	+	<i>N. nova</i>	Probable
#5	TMP-SMX	CNS, Oc, Lung	Cerebral CT scan : multiple abscesses + 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	+	<i>N. shimofuensis</i> <i>/ higoensis</i>	Probable
#7	Pentamidine	CNS, Oc, Lung	Cerebral CT scan : multiple abscesses + Chest CT scan : small lung nodule + pleural condensation	BAL	not made	-	+	<i>N. nova</i>	Probable
#8	Pentamidine	CNS, Lung	Cerebral MRI : 2 large abscesses + Chest CT scan : lung nodule	CSF, BAL, Abscess	+	+(abscess)	+(CSF)	<i>N. nova</i>	Proved
#9	TMP-SMX	CNS, Lung	Cerebral MRI : abscesses + Chest CT scan : lung abscess + lungs nodules	No-biopsy able, BAL not made	not made	not made	not made	-	Possible
#10	Atovaquone	Lung	Chest CT scan : lungs condensations	BAL not made Biopsy not made	not made	not made	not made	-	Possible
#11	TMP-SMX	CNS, Lung, Skin	Cerebral CT scan : abscesses + Body CT scan : lungs condensations + liver abscess	Blood culture	not made	+	not made	<i>N. farcinica</i>	Proved
#12	TMP-SMX	Lung	Chest CT scan : lungs condensations + lungs nodules	BAL	indirect	Few GPB, none identification able	+	<i>N. abscessus</i>	Probable
#13	None	Lung	Chest CT scan : lungs nodules, condensation, abscesses + pleural condensation	Pleural biopsy	indirect	-	+	<i>N. farcinica</i>	Probable
#14	None	CNS	Cerebral CT scan : compressive abscess	CSF	-	-	+	<i>N. spp</i>	Probable
#15	None	Lung	Chest CT scan : lung abscess +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

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

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