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Original article

Diagnosis and management of nocardiosis after bone marrow stem cell transplantation in adults: Lack of lymphocyte recovery as a major contributing factor

Prise en charge diagnostique et thérapeutique de la nocardiose après allogreffe de moelle osseuse chez l'adulte : le déficit de la reconstitution lymphocytaire est un facteur favorisant déterminant

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ABSTRACT

Hematopoietic cell transplantation (HCT) is a curative treatment for hematological malignancies. This therapeutic approach is associated with a profound immune deficiency and an increased rate of opportunistic infections. Nocardiosis is a rare bacterial infection occurring mainly in patients with deficient cell-mediated immunity, such as AIDS patients or transplant recipients. Diagnosis of nocardiosis can be challenging, as signs and symptoms are non-specific. Routine prophylaxis with trimethoprin/sulfamethoxazole (TMP/SMZ) does not prevent the risk of infection. Between May 2001 and December 2009, five cases of nocardiosis were diagnosed from the 366 allogeneic HCT recipients in our centre. Four patients developed a disseminated nocardiosis within the first year after HCT. The fifth patient presented a localized cutaneous nocardiosis. In disseminated cases, median total CD4+ T-cells were below 100 cells/μL. Naive CD4+ CD45RA+/RO− T-cells were almost undetectable. $CD8^+$ T-cells and NK cells were below the normal range and CD19+ B-cell reconstitution was completely deficient. In a localized case, we observed a lack of naive thymic emigrants CD4+ CD45RA+/RO− T-cells.

Mots clés :
Nocardia
Déficit immunitaire
Allogreffe de CSH
Lymphopoïèse
Restauration immunitaire

RÉSUMÉ

L'allogreffe de cellules souches hématopoïétiques (ACSH) est un traitement curatif des hémopathies. Cette thérapeutique est associée à un profond déficit immunitaire et un risque important d'infections opportunistes. La nocardiose est une infection bactérienne rare qui survient préférentiellement chez les patients ayant un déficit de l'immunité cellulaire comme les patients atteints de sida ou les greffés. Le diagnostic est difficile en raison de signes cliniques et biologiques peu spécifique de l'infection. La prophylaxie systématique par cotrimoxazole n'assure pas une protection totale. Cinq cas de nocardioses ont été diagnostiqués entre mai 2001 et décembre 2012 dans notre centre parmi 475 greffes d'ACSH. Quatre cas sur 5 sont des cas de nocardioses disséminées survenant dans les deux premières années de l'allogreffe. Le cinquième cas est une nocardiose localisée cutanée. Parmi les cas de nocardioses

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disséminées, le taux médian de lymphocytes T CD4+ était inférieur à 300/mm^3. Les lymphocytes T CD4+ à phénotype naïf CD45RA+/RO– étaient quasiment indétectables. Les lymphocytes T CD8+ et les cellules Natural Killer étaient majoritairement inférieures aux normes et la reconstitution des lymphocytes B CD19+ complètement inexistante. Concernant le cas de nocardiose localisée, nous avons observé également un profond déficit de la reconstitution des lymphocytes T CD4+ naïfs issus récemment du thymus de phénotype CD45RA+/RO–.

1. Introduction

Allogeneic hematopoietic cell transplantation (AHCT) is associated with a profound immune deficiency and an increased risk of opportunistic infection, which both improve slowly after the transplantation procedure. Innate immunity, including epithelial barriers and phagocytes, typically recovers within weeks after grafting while NK and CD8$^+$ T-cells recover within a few months after AHCT, CD19+ B-cell and CD4+ T-cell recovery is frequently delayed [1]. CD4$^+$ T-cell reconstitution is performed through an early peripheral expansion of graft-derived mature memory T-cells followed by a central output of naive thymic emigrants (NTE). Recovery of NTE (CR45RA+/RO–CD4+ T-cells) is known to decrease the rate of mortality due to infection and essential for a favourable long-term outcome in adults receiving AHCT [2]. This recovery is particularly slow in adult patients with poor thymic function and correlates with the risk of atypical opportunistic infections [1].

Nocardia spp. are Gram-positive bacilli (order of Actinomycetae), ubiquitous soil organisms, with more than 80 species isolated from clinical infections [3]. Nocardiosis is a rare opportunistic infection that mainly affects patients with deficient cell-mediated immunity, such as those with acquired immunodeficiency syndromes or transplant recipients, especially after solid organ transplantation [4]. Detection of *Nocardia* is not routinely included in the work-up of infections after AHCT and nocardiosis is only considered when all other frequent infections have been ruled out. *Nocardia* spp. have rarely been reported after HCT. Only two retrospective series have systematically explored post-HCT nocardiosis and one recent study focuses on the emergence of a new pathogenic species [5,6].

We retrospectively describe diagnosis, management and outcome of nocardiosis after AHCT in our center between May 2001 and December 2012 and search for correlations of these data with immune responses evaluated by phenotypic lymphocyte recovery at the time of infection.

2. Patients and methods

2.1. Patients

We describe five cases of nocardiosis among 475 AHCTs performed in the hematopoietic transplantation unit of our university hospital (Besançon, France) between May 2001 and December 2012. Conditioning regimens and graft verus host disease (GVHD) prophylaxis was adapted to disease status and to each patient's conditions according to local policy and JACIE recommendations (Table 1).

2.2. Infectious supportive care

During the first month following AHCT, all patients were hospitalized in HEPA filtered rooms before discharge. Intravenous antibiotics were administered as needed in febrile hospitalized patients. Infectious prophylaxis (bacterial, viral and fungal) was administered according to the ongoing validated schedules as previously described [7]. Briefly, all patients received either a monthly pentamidine inhalation or oral trimethoprim/sulfamethoxazole (TMP/SMZ 3 times a week). Intravenous polyvalent immunoglobulins were infused (400 mg/kg), when IgG levels were lower than 5 g/L. Oral empiric broad-spectrum antibiotics (ciprofloxacin and amoxicillin + clavulanic acid) began at home in the event of fever above 38 °C. Vaccines were administered according to the validated schedules of the European Bone Marrow Transplantion Group (EBMT): antipneumococcal vaccine began three months after AHCT and pentavalent vaccines (diphtheria, poliomyelitis, tetanus, *Bordetella pertussis* and *Haemophilus*) were administered 6–12 months after transplantation [7].

2.3. Standard laboratory methods

Cytomegalovirus (CMV) replication (PCR detection) and aspergillosis markers (platelia antigenemia and PCR detection) were assessed weekly until immunosuppression was withdrawn as previously described [8]: fungal infections were classified according to the international consensus [9]. The following analyses were performed when patients presented a septic syndrome: blood sample cultures, aspergillus antigenemia, CMV PCR, adenovirus PCR, CRP, chest radiography, computed tomography and urine analysis. The aim of this complete biological work-up was to rule out frequent opportunistic infections quickly.

2.4. Specific microbiological identification of Nocardia

Nocardia can grow in all standard cell culture media. A specific cell culture medium (called Bromcresol Purple, BCP, Paris, France) containing lactose speeds up *Nocardia* growth (1 day) [10,11]. Growth of *Nocardia* can take 2–5 days and colonies appear chalky white if producing aerial hyphae. In our center, *Nocardia* detection is centralized in Lyon (*Observatoire français des nocardioses*, http://ofn.univ-lyon1.fr/); after presumptive identification, the reference laboratory confirms the identification. All isolates were identified by phenotyping and genotyping. The isolates were biochemically identified to species level by the following tests: hydrolysis of casein, tyrosine, xanthine, and hypoxanthine; growth at 45 °C; arylsulfatase production; and antibiotic susceptibility testing performed by disk diffusion method according to the CLSI (Clinical and Laboratory Standards Institute) standards M24-A2 guidelines [12]. The antibiotic disks used were amoxicillin (25 μg), amoxycillin/clavulanic acid (20/10 μg), ampicillin (10 μg), ceftriaxone (30 μg), cefuroxime (30 μg), cefotaxime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), linezolid (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), amikacin (30 μg), gentamicin (15 μg), tobramycin (10 μg), imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), tigecycline (15 μg), moxifloxacin (5 μg), minocycline (30 μg), doxycycline (30 μg), vancomycin (30 μg), piperacillin/tazobactam (75/10 μg), rifampin (30 μg) and erythromycin (15 μg) (Bio-Rad, Marnes-la-Moquette, France); plates were

Table 1
Pre-transplant donor (D) and recipient (R) clinical features.

Pts	Age (years) D/R	Sex D/R	CMV D/R	Disease status	Diagnosis	CD34+ cells (10^6/Kg)	Regimens	GVHD prophylaxis	Source of graft	Graft type
1	44/56	M/M	+/–	CR	AREB1	1.29	Bus CY	CsA-MTX	Unrelated donor	BM
2	54/57	F/M	+/+	PD	AML	1.83	Bus F	CsA +ATG	Familial donor	BM
3	49/56	M/M	–/–	PR	Myeloma	1.98	F Mel	CsA-MTX	Familial donor	BM
4	41/36	F/M	+/–	CR	AML	2.94	TBI CY	CsA-MTX	Familial donor	BM
5	40/34	F/M	+/–	CR	AML	2.94	TBI CY	CsA-MTX	Familial donor	BM

Pts: patients; M: male; F: female; CMV: cytomegalovirus; PD: progression disease; CR: complete response; PR: partial response; AML: acute myeloid leukemia; AREB1: refractory anaemia with blast excess; Bus: busilvex; CY: cyclophosphamide; F: fludarabine; Mel: melphalan; CsA: cyclosporine; MTX: methotrexate; ATG: anti human thymoglobulins; TBI: total body irradiation; BM: bone marrow; D/R: recipient/donor of allogeneic bone marrow transplantation.