J Clin Immunol (2017) 37:36–41
DOI 10.1007/s10875-016-0342-x

ORIGINAL ARTICLE

Early Identification of Lung Fungal Infections in Chronic Granulomatous Disease (CGD) Using Multidetector Computer Tomography

Maria Pia Bondioni[1,2] · Vassilios Lougaris[3] · Giuseppe Di Gaetano[2] · Tiziana Lorenzini[3] · Annarosa Soresina[3] · Francesco Laffranchi[2] · Diego Gatta[4] · Alessandro Plebani[3]

Received: 1 March 2016 / Accepted: 7 October 2016 / Published online: 21 October 2016

Abstract
Purpose The purpose of this study is to evaluate the possibility of early detection of pulmonary fungal infections by lung CT scan in chronic granulomatous disease (CGD).
Methods A retrospective study on 14 patients affected with CGD for a total of 18 infectious episodes was performed. Revision of clinical data and CT scan analysis before and after treatment was performed.
Results The presence of lung nodules <30 mm was evaluated in 18 infectious episodes in 14 patients. A total of 125 nodules in 18 CT scans were identified. Identification of the infectious agent through biopsy and in vitro culture resulted positive only in 3/18 cases. The remaining cases received clinical/radiologic diagnosis of suspected pulmonary fungal infection. In all cases, the introduction of empirical antifungal treatment resulted in reduction in size or complete resolution of the pulmonary lung nodules in all patients affected with CGD.
Conclusions Lung CT scan allows for early detection of pulmonary fungal infection in CGD. Pulmonary nodules (<30 mm), single or multiple, uni- or bilateral, with or without a halo sign may represent the first radiologic sign of pulmonary fungal infection in CGD.

Keywords Chronic granulomatous disease (CGD) · fungal infections · lung CT scan

Abbreviations

CGD	chronic granulomatous disease
CT	computed tomography
NADPH	nicotinamide adenine dinucleotide phosphate
NBT	nitrotetrazolium blue chloride
DHR123	dihydrorhodamine 123
CRP	C-reactive protein
IPA	invasive pulmonary aspergillosis

Introduction

Chronic granulomatous disease (CGD) is a rare inborn phagocyte disorder characterized by defects in the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex [1, 2]. Both X-linked and autosomal forms of transmission have been described, with the former being the most frequent (around two thirds of cases) [1]. Affected patients present an increased susceptibility to bacterial and fungal infections, granulomata formation, and autoimmune manifestations [3–5]. Bacteria, such as *Staphylococcus aureus* and *epidermidis*, *Burkholderia cepacia*, *Escherichia coli*, *Salmonella*, *Klebsiella*, *Serratia marcescens*, *Pseudomonas* species, and fungi, mainly *Aspergillus* species and *Candida*, are the most frequently isolated pathogens. Infections may affect the lungs, skin, bone, and lymph nodes [6].

Maria Pia Bondioni and Vassilios Lougaris contributed equally to this work.

✉ Maria Pia Bondioni
mariapiabondioni@gmail.com

[1] Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy

[2] Pediatric Radiology Unit, ASST Brescia, Piazzale Spedali Civili 1, Brescia 25123, Italy

[3] Pediatrics Clinic and Institute for Molecular Medicine A. Nocivelli, Department of Clinical and Experimental Sciences, University of Brescia, Piazzale Spedali Civili 1, Brescia 25123, Italy

[4] Unit of Pneumology, Ospedale di Esine, Brescia, Italy

The introduction of antibacterial (cotrimoxazole) and antifungal (mainly itraconazole) chemoprophylaxis, together with earlier diagnosis, has allowed for a better long-term survival [7, 8]. However, the clinical history of affected patients remains still complicated by recurrent bacterial and fungal infections, frequently invasive ones. In the last years, bone marrow transplantation and gene therapy are becoming promising therapeutic strategies for patients with CGD [1, 3, 9].

Fungal infections, mainly caused by *Aspergillus* spp., account for an important part of the infectious complications in CGD [8, 10]. The infection is generally localized in the lung, following inhalation of spores, and may manifest as pulmonary aspergilloma or invasive pulmonary aspergillosis [1, 4, 8, 10–12]. Unfortunately, current diagnostic approaches, including laboratory testing and bioptic examination, do not always allow for definite diagnosis, especially in the early stages of infection. Consequently, these types of infections may result in long periods of hospitalization with important complications for affected patients such as pulmonary dissection and disseminated infection. Early diagnostic and/or predictive parameters for pulmonary fungal infections in CGD have not been identified yet. We decided to investigate the clinical significance in terms of fungal infection of the presence of small nodules (<30 mm) in lung CT scans, in the diagnosis and follow-up of suspected pulmonary fungal infection in patients affected with CGD, especially since available data in the literature regarding this issue are scarce. Our data show that the presence of small nodules (<30 mm) in lung CT scans in CGD are strongly suggestive of fungal infections allowing for an early and appropriate treatment, despite uninformative laboratory data.

Materials and Methods

Patients

Fourteen patients (female/male ratio 2:12) affected with CGD followed at a single center between 1990 and 2014 were included in this study. Diagnosis of CGD was based on defective NBT test or pathologic DHR123 testing. Mean age at diagnosis was 21.28 months (range, 1–84 months). Four out of the fourteen patients presented with the autosomal recessive form of CGD, while the remaining ten suffered from the X-linked one. All patients were under antifungal prophylaxis with itraconazole (except for three patients diagnosed upon the first lung CT scan) and antibiotic prophylaxis with cotrimoxazole (except for three patients diagnosed upon the first lung CT scan and two patients that presented adverse reaction to cotrimoxazole and were switched to cefpodoxime). The patients' inclusion criteria were the presence of nodules (abnormalities) at lung CT scan suggestive of fungal infections despite the lack of microbiologic confirmation. Lung CT scan was performed because of persistence of fever, cough, and thoracic pain, irresponsive to wide spectrum antibiotic therapy. Clinical and laboratory findings at presentation included fever in 6 out of 18 cases (33.33 %), cough in 1 out of 18 cases (5.55 %), thoracic pain in 2 out of 18 cases (11.11 %), leukocytosis (white blood cell count >11,000/mm^3) in 7 out of 18 cases (38.88 %), neutrophilia (absolute neutrophil count >7700/mm^3) in 6 out of 18 cases (33.33 %), and elevated C-reactive protein (CRP) in 17 out of 18 cases (94.44 %). Eight patients, during follow-up, presented one lung fungal infection, while the remaining six patients presented at least two or more fungal lung infections with different radiologic presentation for each infectious episode. Laboratory confirmation of fungal infection in the presence of lung nodules on the CT scan was achieved only in 5 cases (*Aspergillus spp.* in 3 cases, *Scedosporium spp.* in 2 cases); in the remaining 13 cases presenting with nodules, an etiologic agent was not identified. Treatment with voriconazole was undertaken in 8 cases (8/18; 44.44 %), with amphotericin B in 8 cases (8/18; 44.44 %), and posaconazole in the remaining 2 cases (2/18; 11.11 %). All patients received contemporaneous broad spectrum antibiotic treatment. The total time of antifungal treatment for the lung infection was variable depending on the type of lesion and the clinical response (median 2.2 months; range 1 to 5 months). The interval between CT scans (median 2.2 months; range 1 to 5 months) was decided on clinical basis (response to treatment) and on the associated lung lesions such as masses, lymphadenopathy, parenchymal consolidation, excavation, and pleural effusion.

CT Scan

Chest CT examinations were performed with a Somatom Plus 4 scanner (Siemens Medical Systems, Erlangen, Germany) before 2003 and with a 6-slice MDCT scanner (Emotion 6, Siemens Medical Systems, Erlangen, Germany) or 16-slice MDCT scanner (Somatom Sensation 16, Siemens Medical Systems, Erlangen, Germany) after 2003, as previously described [13]. In some cases, and based on the suspicion of infiltrating pulmonary mass, CT scan was performed with contrast media administration using an antecubital vein at the dosage of 2 ml/kg of iodinated CM. The second CT scan (after treatment) was performed without CM administration for all patients.

CT scans were evaluated for all 14 affected patients (18 infectious episodes) in order to characterize and monitor lung nodules before and after treatment and correlate these findings with the clinical outcome.

In our study, nodules were defined as a rounded or irregular opacity, well or poorly defined, with or without a halo sign, and measuring up to 3 cm in diameter, according to the literature data [14]. Nodules were evaluated based on their number: single, sporadic, or multiple (defined arbitrally as 1 or <5

or >5 nodules, respectively); their distribution (unilateral, bilateral); their structure (solid or with excavation); and their dimensions (>5 mm, >5–30 mm, or both).

Results

Lung Nodules in CGD

Lung CT scan evaluation revealed the presence of 125 nodules in 18 scans.

Nodules were sporadic in 11/18 cases, while they were multiple in 5/18 cases and single in 2/18 cases. They showed a bilateral presentation in 11/18 cases, while they were unilateral in 7/18 cases. The margins of these nodules were regular in 9/18 cases and irregular in 4/18 cases, while a mixed pattern was observed in 5/18 cases. The dimensions of the lung nodules in CGD was less than 5 mm in 2/18 cases, between 5 and 30 mm in 4/18 cases while nodules with mixed dimensions were observed in 12/18 cases (Table 1). A halo sign was present in 8/18 cases (two of which had an etiological confirmation of fungal infection) and absent in the remaining ones (10/18). An air crescent sign was found only in one case (1/18) and was associated with the bigger nodular lesion (14 mm).

The heterogeneous features of the nodules observed in the lung CT scans of the index patients prompted us to divide them in single, sporadic, or multiple and better evaluate their characteristics before and after antifungal treatment, in order to confirm their fungal origin.

Single Nodules

Single nodules were found in 2 cases before treatment (2/18 cases; 2/125 nodules). The margins of these nodules were regular in both cases (2/2). The dimensions of the lung nodules were 15 and 18 mm. The latter was accompanied by the presence of a halo sign. An air crescent sign was not present. Upon treatment, CT scan revealed the resolution of the 15-mm nodule and the reduction of diameter for the second one (from 18 to 14 mm), while a halo sign was not detectable (Fig. 1).

Sporadic Nodules

Sporadic nodules were present in 11/18 cases (37/125 nodules). The margins of these nodules were regular in 6/11 cases and irregular in 5/11 cases. The dimensions of the lung nodules ranged from 2 to 30 mm. A halo sign was present in 4/11 cases. An air crescent sign was not present. Upon treatment, CT scan revealed complete resolution in 5/11 cases and reduction of diameter in 4/11 cases (Fig. 2), and no halo sign was evident. In one patient with 2 nodules (6 and 7 mm), no changes in diameter were detected. Finally, in one patient, the nodules were increased in volume; however, the patient was not adherent to the prophylaxis regimen.

Table 1 Radiologic features of lung nodules in 14 CGD patients at presentation of suspected/probable fungal infection (total number of CT scans $n = 18$)

	Numbers (percentages)
Presentation	
Single	2/18 (11.11)
Sporadic	10/18 (55.55)
Multiple	6/18 (33.33)
Distribution	
Unilateral	11/18 (61.11)
Bilateral	7/18 (38.89)
Margins	
Regular	9/18 (50.00)
Irregular	5/18 (27.77)
Both	4/18 (22.22)
Dimensions	
<3 mm	0/18 (00.00)
>3 and <30 mm	4/18 (22.22)
Both	14/18 (77.77)

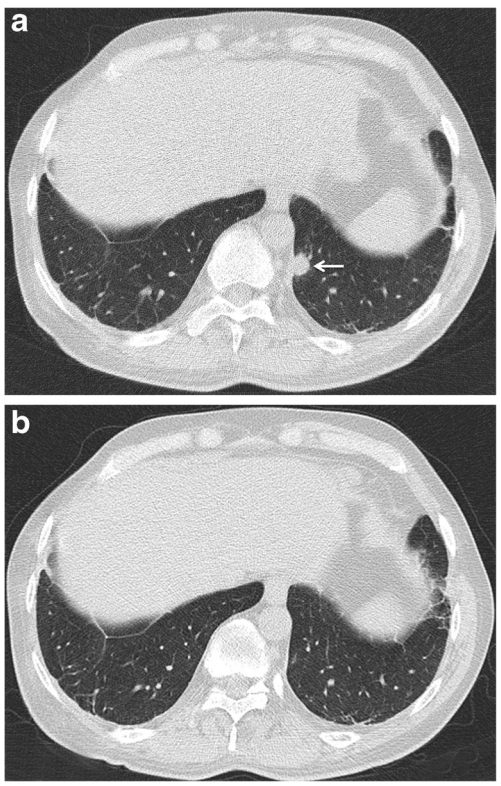

Fig. 1 Single nodule in lung CT scan in CGD. **a** CT scan with lung algorithm on the axial plane: before treatment, a single, well-defined nodule in the posterior segment of the inferior left lobe (*arrow*) can be seen. **b** Upon medical treatment, the nodule is no longer visible. Some peripheral, bilateral scars can be detected

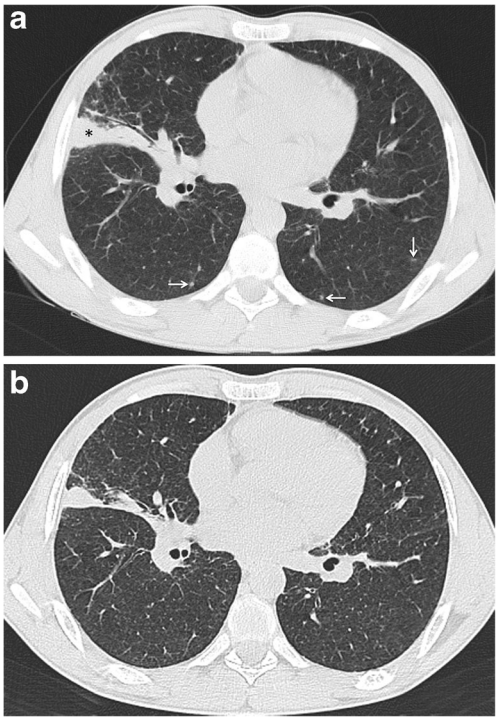

Fig. 2 Sporadic nodules in lung CT scan in CGD. **a** CT scan with lung algorithm on the axial plane: small round, well-delimited nodules can be detected (*arrows*) in both lungs. In the right lower lobe, an area of parenchymal consolidation co-exists with air bronchogram (*black asterisk*). **b** Upon medical treatment, the nodules are no longer detectable

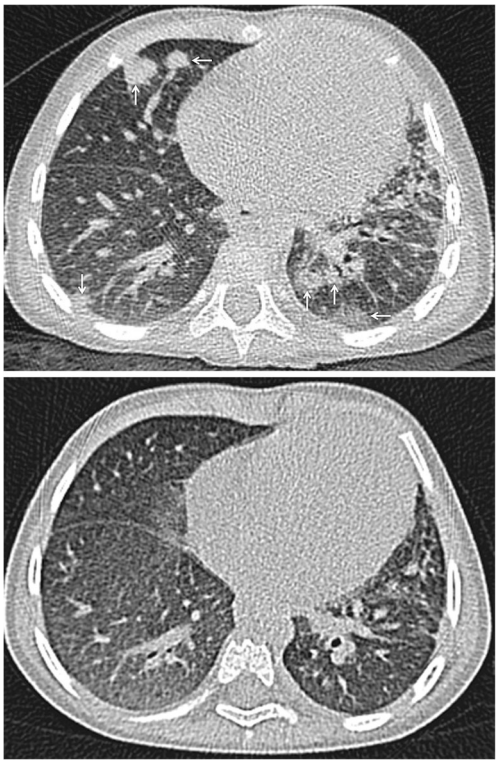

Fig. 3 Multiple nodules (IPA) in lung CT scan in CGD. **a** CT scan with lung algorithm on the axial plane: in both lungs, multiple parenchymal opacities (*arrows*) with a mixed pattern can be seen. **b** Upon medical treatment, the nodules are no longer detectable

Multiple Nodules

Multiple nodules were present in 5/18 cases (86/125 nodules). The margins of these nodules were regular in 1/5 cases and with a mixed pattern in 4/5 cases. The dimensions of the lung nodules ranged from 2 to 27 mm. Halo sign was present in 4/5 cases. An air crescent sign was present in 3/5 cases. Upon treatment, CT scan revealed complete resolution in 2/5 cases and reduction of diameter in 3/5 cases, while no halo sign was anymore evident. Multiple nodules were associated with masses in 4 out of 5 cases similarly to invasive pulmonary aspergillosis (IPA) [15] (Fig. 3).

Summarized data regarding all nodules' characteristics (number and size) before and after treatment are shown (Fig. 4). All nodules, with or without a microbiologically defined etiology, showed a similar reduction/resolution pattern upon treatment.

Discussion

Chronic granulomatous disease (CGD) is a rare inborn disorder characterized by defective granulocyte phagocytosis due to impaired NADPH activity [1, 2]. The functional deficiency of granulocytes results in complex infectious phenotypes sustained by atypical pathogens, such as *Candida spp.*, *Aspergillus spp.* and other fungi, as well as bacterial infections sustained by *Staphylococcus spp.*, *E. coli*, *Salmonella*, *Klebsiella*, *Serratia*, and others. These infections may involve

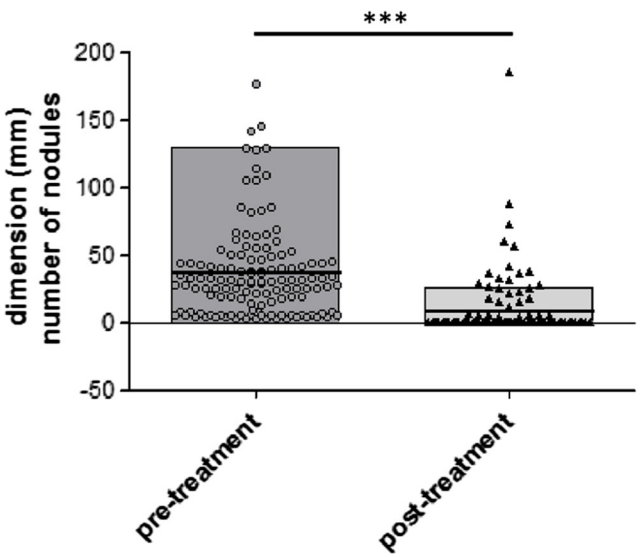

Fig. 4 Summarized data of numbers and dimensions for nodular lesions on lung CT scans before and after antifungal treatment in CGD. **a** Number (*gray box*) and dimensions (*dots*) of nodular lesions before (*left bar*) and after treatment (*right bar*); the *horizontal line* depicts the median number of the nodular lesions. Statistical analysis was performed using the paired *t* test both for numbers and dimensions of nodules (***$p < 0.0001$)

any organ including the skin, lungs, intestine, urinary tract, liver, bone, and CNS (central nervous system) [3–6].

Lung involvement in CGD during fungal infections is a frequent complication for affected patients [10–12]. Radiological findings at lung CT scans may include parenchymal consolidations with or without an air crescent sign, masses with or without thoracic wall involvement, mediastinal lymphadenopathy, pleural effusion, and tree-in-bud pattern [6, 11, 12, 14, 16–23]. In many cases, nodular lesions may be identified at CT scans in the presence or absence of the above mentioned lesions [16, 18, 20–23]. Nonetheless, these nodular lesions, especially when of small size or in limited number, are difficult to interpret and relevant literature data on their potential fungal origin in CGD patients are scarce. In addition, early phase lung fungal infections in CGD are not easy to diagnose on a laboratory basis given the very low yield of fungi isolation from lung biopsies. In this study, we decided to study small nodules (<30 mm) in lung CT scans from CGD patients with suspected fungal infection and evaluate their response to systemic antifungal treatment, in order to confirm their fungal origin.

A total of 125 small nodules were identified at the initial CT scans during 18 infectious episodes in 14 CGD patients. While in 5 cases, small nodules were multiple, in the remaining 13 cases, nodules were either single (2) or sporadic (11) small nodules. Interestingly, single or sporadic nodules were of smaller dimensions when compared to the multiple nodular presentation, probably due to different stages of the fungal infection. Margins were either regular or irregular, with the latter pattern is more frequently observed in multiple nodules cases. The CT scan upon endovenous antifungal treatment showed a remarkable modification of the small nodular lesions, with either complete resolution or reduction in size (all) and/or number (in the case of sporadic o multiple nodules) in all patients confirming their fungal origin. Multiple small nodules in the CGD cases were associated with masses in 4/5 cases, resembling invasive pulmonary aspergillosis (IPA) [15]. Nonetheless, even these nodules behaved similarly to single or sporadic small nodules upon endovenous antifungal treatment. Of note, this resolution/reduction pattern upon endovenous antifungal treatment was observed also in the smaller nodular lesions, even below 10 mm, irrespective of the type of presentation (single, sporadic, or nodular with the exception of one). These findings are novel and of importance since small lung nodules have not been studied extensively yet in CGD and since our data strongly suggest that they may be considered as early radiological signs for lung fungal infection in CGD.

In conclusion, we describe the first extensive study of small nodular lesions in CGD patients with suspected pulmonary fungal infections. We present strong evidence arguing that small nodular lesions in CGD, single or numerous, with regular or irregular margins, with or without a halo sign, are suggestive of pulmonary fungal infection. CT scans may allow therefore for early and timely diagnosis in fungal lung disease in CGD and affected patients may benefit from appropriate medical treatment.

Acknowledgments We would like to thank the patients, their families, and the nurses for all their help and support.

Authors' Contributions Maria Pia Bondioni, Vassilios Lougaris, and Alessandro Plebani conceived the study and wrote the protocol. Maria Pia Bondioni, Giuseppe Di Gaetano, and Francesco Laffranchi analyzed the CT scan data. Vassilios Lougaris and Tiziana Lorenzini studied the clinical data. Annarosa Soresina followed the patients and offered clinical data. Maria Pia Bondioni, Vassilios Lougaris, and Alessandro Plebani wrote the manuscript. All authors read and approved the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Funding None.

References

1. Chiriaco M, Salfa I, Matteo GD, Rossi P, Finocchi A. Chronic granulomatous disease: clinical, molecular and therapeutic aspects. Pediatr Allergy Immunol. 2015. doi:10.1111/pai.12527.
2. Holland SM. Chronic granulomatous disease. Hematol Oncol Clin North Am. 2013;27(1):89–99. doi:10.1016/j.hoc.2012.11.002. viii.
3. Kang EM, Marciano BE, DeRavin S, Zarember KA, Holland SM, Malech HL. Chronic granulomatous disease: overview and hematopoietic stem cell transplantation. J Allergy Clin Immunol. 2011;127(6):1319–26. doi:10.1016/j.jaci.2011.03.028. quiz 1327-8.
4. Falcone EL, Holland SM. Invasive fungal infection in chronic granulomatous disease: insights into pathogenesis and management. Curr Opin Infect Dis. 2012;25(6):658–69. doi:10.1097/QCO.0b013e328358b0a4.
5. Seger RA. Advances in the diagnosis and treatment of chronic granulomatous disease. Curr Opin Hematol. 2011;18(1):36–41. doi:10.1097/MOH.0b013e32834115e7.
6. Towbin AJ, Chaves I. Chronic granulomatous disease. Pediatr Radiol. 2010;40(5):657–68. doi:10.1007/s00247-009-1503-3. quiz 792-3.
7. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. PLoS One. 2009;4(4), e5234. doi:10.1371/journal.pone.0005234.
8. Martire B, Rondelli R, Soresina A, Pignata C, Broccoletti T, Finocchi A, Rossi P, Gattorno M, Rabusin M, Azzari C, Dellepiane RM, Pietrogrande MC, Trizzino A, Di Bartolomeo P, Martino S, Carpino L, Cossu F, Locatelli F, Maccario R, Pierani P, Putti MC, Stabile A, Notarangelo LD, Ugazio AG, Plebani A, De Mattia D; IPINET. Clinical features, long-term follow-up and outcome of a large cohort of patients with chronic granulomatous disease: an Italian multicenter study. Clin Immunol. 2008 Feb;126(2):155-64.
9. Kaufmann KB, Chiriaco M, Siler U, Finocchi A, Reichenbach J, Stein S, et al. Gene therapy for chronic granulomatous disease:

current status and future perspectives. Curr Gene Ther. 2014;14(6):447–60.
10. Marciano BE, Spalding C, Fitzgerald A, Mann D, Brown T, Osgood S, et al. Common severe infections in chronic granulomatous disease. Clin Infect Dis. 2015;60(8):1176–83. doi:10.1093/cid/ciu1154.
11. Salvator H, Mahlaoui N, Catherinot E, Rivaud E, Pilmis B, Borie R, et al. Pulmonary manifestations in adult patients with chronic granulomatous disease. Eur Respir J. 2015;45(6):1613–23. doi:10.1183/09031936.00118414.
12. Mahdaviani SA, Mohajerani SA, Rezaei N, Casanova JL, Mansouri SD, Velayati AA. Pulmonary manifestations of chronic granulomatous disease. Expert Rev Clin Immunol. 2013;9(2):153–60. doi:10.1586/eci.12.98.
13. Bondioni MP, Soresina A, Lougaris V, Gatta D, Plebani A, Maroldi R. Common variable immunodeficiency: computed tomography evaluation of bronchopulmonary changes including nodular lesions in 40 patients. Correlation with clinical and immunological data. J Comput Assist Tomogr. 2010;34(3):395–401.
14. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Muller NL, Remy J. Fleischner Society: glossary of terms for thoracic imaging. Radiology. 2008;246:697–722.
15. Lim C, Seo JB, Park SY, Hwang HJ, Lee HJ, Lee SO, et al. Analysis of initial and follow-up CT findings in patients with invasive pulmonary aspergillosis after solid organ transplantation. Clin Radiol. 2012;67(12):1179–86.
16. Godoy MC, Vos PM, Cooperberg PL, Lydell CP, Phillips P, Müller NL. Chest radiographic and CT manifestations of chronic granulomatous disease in adults. AJR Am J Roentgenol. 2008;191(5):1570–5. doi:10.2214/AJR.07.3482.
17. Moghtaderi M, Kashef S, Rezaei N. Interstitial lung disease in a patient with chronic granulomatous disease. Iran J Pediatr. 2012;22(1):129–33.
18. Khalilzadeh S, Bloorsaz MR, Mansouri D, Baqhaie N, Hakimi S, Velayati AA. Clinical and radiological aspects of chronic granulomatous disease in children: a case series from Iran. Iran J Allergy Asthma Immunol. 2006;5(2):85–8.
19. Matsuzono Y, Togashi T, Narita M, Taguchi Y, Miura S. Pulmonary aspergillosis and pseudosequestration of the lung in chronic granulomatous disease. Pediatr Radiol. 1995;25(3):201–3.
20. Chusid MJ, Sty JR, Wells RG. Pulmonary aspergillosis appearing as chronic nodular disease in chronic granulomatous disease. Pediatr Radiol. 1988;18(3):232–4.
21. de Sévaux RG, Kullberg BJ, Verweij PE, van de Nes JA, Meis JF, van der Meer JW. Micrtogranulomatous aspergillosis in a patients with chronic granulomatous disease: cure with voriconazole. Clin Infect Dis. 1998;26(4):996–7.
22. Sierre S, Lipsich J, Santos P, Hernandez C, Siminovich M, Oleastro M, et al. Pulmonary fungal infections diagnosis in chronic granulomatous disease patients. Pediatr Pulmonol. 2007;42(9):851–2.
23. Agudelo-Flórez P, Prando-Andrade CC, López JA, Costa-Carvalho BT, Quezada A, Espinosa FJ, et al. Chronic granulomatous disease in Latin American patients: clinical spectrum and molecular genetics. Pediatr Blood Cancer. 2006;46(2):243–52.