

- 4 Reynaert NL, Wouters EF, Janssen-Heininger YM. Modulation of glutaredoxin-1 expression in a mouse model of allergic airway disease. *Am J Respir Cell Mol Biol* 2007; 36: 147–151.
- 5 Brightling CE. Sputum induction in asthma: a research technique or a clinical tool? *Chest* 2006; 129: 503–504.
- 6 Nagai K, Betsuyaku T, Konno S, *et al.* Diversity of protein carbonylation in allergic airway inflammation. *Free Radic Res* 2008; 42: 921–929.
- 7 Peltoniemi M, Kaarteenaho-Wiik R, Saily M, *et al.* Expression of glutaredoxin is highly cell specific in human lung and is decreased

- by transforming growth factor- β *in vitro* and in interstitial lung diseases *in vivo*. *Hum Pathol* 2004; 35: 1000–1007.
- 8 Lundberg M, Fernandes AP, Kumar S, *et al.* Cellular and plasma levels of human glutaredoxin 1 and 2 detected by sensitive ELISA systems. *Biochem Biophys Res Commun* 2004; 319: 801–809.
- 9 Aesif SW, Anathy V, Guala AS, *et al.* Ablation of glutaredoxin-1 attenuates lipopolysaccharide-induced lung inflammation and alveolar macrophage activation. *Am J Respir Cell Mol Biol* 2011; 44: 491–499.

DOI: 10.1183/09031936.00115212

A 61-year-old female with chronic cough and purulent sputum

To the Editor:

A 61-yr-old Caucasian female was referred to our outpatient clinic for persistent cough, right scapular pain and purulent sputum for the past 9 months.

Her past medical history was consistent with a diagnosis of pneumonia almost 20 yrs previously. She reported no allergies and was not taking any medications at home. She lived in an urban setting working as a hairdresser, and had no pets at home. She had received all the standard vaccinations based on the Italian law, including diphtheria, smallpox, tetanus, hepatitis A and typhus. She was married and had two daughters in good health. She went on vacation to Cuba 2 yrs previously, India (Rajasthan) 1 yr previously and Portugal 6 months ago.

Her history of present illness was consistent with cough and mucopurulent sputum without fever for 9 months prior to visiting our clinic. She reported a scapular right pain that had occurred 15 days prior to admission to the hospital which was not related to positioning or breathing. A negative chest radiograph had been performed 3 months prior to the visit, and at that point she was treated with oral prednisone (25 mg every 24 h for 15 days) with no evidence of clinical improvement. Finally, she underwent a chest computed tomography (CT) scan with the evidence of lung opacities with enhancement in the medium lobe and in the lingual (fig. 1a).

At the time she was referred to our outpatient clinic (Respiratory Clinics, Respiratory Department, San Gerardo Hospital, Monza, Italy) she was complaining of respiratory symptoms including cough and purulent sputum, while the physical examination was completely unremarkable. A chest examination was negative and she had did not have skin or pharyngeal lesions. A tuberculin test was performed and the result was negative. Blood examinations were obtained that showed a white blood cell count of $5.510 \text{ cell} \cdot \mu\text{L}^{-1}$ and haemoglobin of $13 \text{ g} \cdot \text{dL}^{-1}$, with no evidence of anti-HIV antibodies.

She underwent bronchoscopy which showed mucopurulent secretions from all the lobar bronchi. The bronchoalveolar lavage (BAL) fluid showed 1,280,000 cells in a 25-mL sample, with 29% macrophages (without hemosiderin pigment), 68% neutrophils, 1% eosinophils, 2% of lymphocytes and <1% of histiocytes

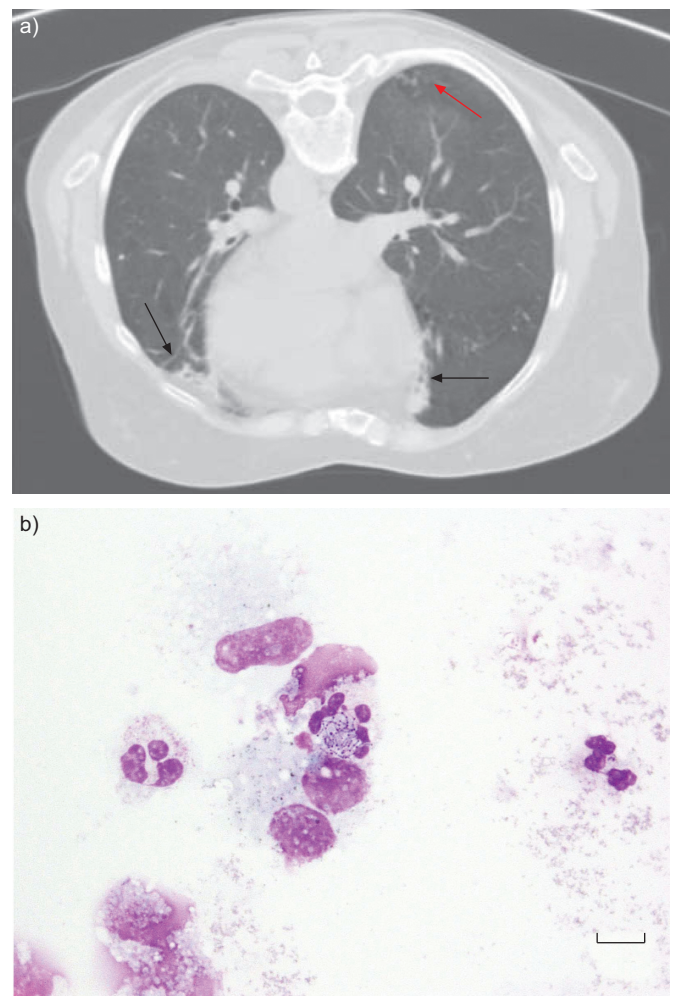


FIGURE 1. a) Chest computed tomography scan showing lung opacities in the medium lobe and in the lingula with bronchiectasis (black arrows). A “tree-in-bud” pattern is also present in the depending areas (red arrow). b) Bronchoalveolar lavage fluid after May–Grünwald Giemsa staining. Several rod-shaped bacteria with irregular staining and “Chinese letters” arrangement of *Corynebacterium* spp. are visible inside a neutrophil. Scale bar=500 μm .

TABLE 1 Clinical manifestations associated with *Corynebacterium diphtheriae* (*gravis*, *mitis*, *intermedius* and *belfanti*) according to toxigenic status

Status	Clinical manifestation
Toxicogenic strain	Respiratory diphtheria [#]
	Pharyngeal, tonsillar or nasal pseudomembranes
	Cutaneous diphtheria [#]
Non-toxicogenic strain	Skin lesion with necrosis and/or pseudomembranes
	Pharyngitis (without pseudomembranes)
	Endocarditis
	Septic arthritis
	Other non-specific types of infections

[#]: can be associated with systemic toxicity, myocarditis or polyneuropathy.

plurinuclates. Cytologic analysis for the detection of neoplastic cells was negative. Evidence of bacteria in the cytoplasm of neutrophils was noted. The result of BAL Gram stain is shown in figure 1b. Culture of the BAL yielded pure growth of *Corynebacterium diphtheriae*. Other microbiological tests including cultures for fungi, microscopic examination for acid-fast bacilli and PCR for the detection of *Mycobacterium tuberculosis* were all negative.

The isolate was sent to the Reference Centre for Diphtheria at the Istituto Superiore di Sanità in Rome, Italy, in order to confirm the species and the toxigenicity status of the isolate. The isolate was confirmed within 1 h as a non-toxicogenic *C. diphtheriae* by a novel combined real-time PCR assay that allows the simultaneous identification of the species and of the toxigenic status [1]. A lack of the diphtheria toxin gene was also confirmed by classical PCR amplification. The strain was biotyped as *C. diphtheriae* biotype *belfanti* using the API Coryne kit (bioMérieux, Lyon, France). Antibiotic susceptibility was performed by E-test (bioMérieux, Marcy-l'Étoile, France): the strain was susceptible to all the antibiotics tested including penicillin, ceftriaxone, ciprofloxacin, erythromycin and gentamycin. By multilocus sequence typing, the isolate was ST234, a single locus variant of ST42.

The patient was treated with 1 g amoxicillin/clavulanate, three times a day for 16 days. 7 days after the initiation of the antibiotic treatment the patient recovered from all the symptoms. She was also treated with azithromycin 500 mg·day⁻¹ and clindamycin 600 mg three times a day for 15 days.

10 days after the initiation of antibiotic therapy, the patient, her husband and her daughters underwent a pharyngeal swab to detect *C. diphtheriae* and results were all negative. 2 months after the diagnosis, the patient underwent bronchoscopy and BAL cultures were all negative. Finally, a new chest CT scan was performed and all the lesions seen on the previous CT scan were reduced in diameter.

We reported a case of chronic pneumonia sustained by a non-toxicogenic *C. diphtheriae* biotype *belfanti* in an Italian immunocompetent female. This is the first report of a low respiratory tract infection sustained by a non-toxicogenic strain of *C. diphtheriae* biotype *belfanti*. The isolate belongs to the novel ST234, a single locus variant of ST42, which has been found in the USA and

Germany. ST42 and its variants are associated with non-toxicogenic *C. diphtheriae* biotype *belfanti* only [2].

A resurgence of diphtheria was reported during the 1990's, especially in the newly independent states of the former Soviet Union due to a reduction of the vaccination coverage. In the rest of Europe only a few cases of diphtheria are reported each year [3]. Conversely, the incidence of non-toxicogenic *C. diphtheriae* strains is increasing [4]. Non-toxicogenic strains can be isolated from different types of infection, such as skin lesions, pharyngitis, bacteremia and endocarditis, as well as in patients with pneumonia, although this is a rare occurrence [5–7]. Immunisation against diphtheria toxin does not protect from the infectivity of non-toxicogenic strains, supporting the hypothesis that *C. diphtheriae* may have additional virulence factors [8]. Most non-toxicogenic *C. diphtheriae* strains causing invasive diseases belong to biotypes *gravis* and *mitis*, while biotypes *intermedius* and *belfanti* are less common [7]. Clinical manifestations associated with *C. diphtheriae* infections are depicted in table 1, according to toxigenic status.

Our case is of some interest for several reasons: 1) the development of chronic pneumonia; 2) the presence of a non-toxicogenic strain; and 3) the absence of severe systemic manifestation. HONMA *et al.* [9] reported a similar case of a Japanese female with amyotrophic lateral sclerosis who suffered from pneumonia sustained by a non-toxicogenic *C. diphtheriae* *mitis*, undergoing tracheostomy and mechanical ventilation because of a respiratory distress. In our case, symptoms developed during a long period of time and there were no life-threatening events.

Different speculations could be generated by our report. Particularly, one could argue if *C. diphtheriae* was the pathogen responsible for those symptoms, or a super infection/colonisation. However, the antibiotic therapy directed to that pathogen led to a positive outcome. The real incidence of infection sustained by non-toxicogenic strains of *C. diphtheriae* is unknown, but we can speculate that the real incidence is underestimated because broad-spectrum antibiotics are currently given empirically to patients.

Luca Bilucaglia*, **Stefano Aliberti***, **Egidio Franco Viganò[#]**, **Annalisa Pantosti[†]**, **Monica Monaco[†]**, **Alessandra Ciervo[†]**, **Fabiola Mancini[†]**, **Paola Faverio*** and **Alberto Pesci***

*Dipartimento di Medicina Clinica e Prevenzione, University of Milan-Bicocca, Clinica Pneumologica, AO San Gerardo, [#]Microbiology Dept, AO San Gerardo, Monza, and [†]Dept of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy.

Correspondence: S. Aliberti, Dipartimento di Medicina Clinica e Prevenzione, University of Milan-Bicocca, Clinica pneumologica, AO San Gerardo, Via Pergolesi 33, 20052, Monza, Italy. E-mail: stefano.aliberti@unimib.it

Statement of Interest: None declared.

REFERENCES

- Mancini F, Monaco M, Pataracchia M, *et al.* Identification and molecular discrimination of toxigenic and non-toxicogenic diphtheria *Corynebacterium* strains by combined real time PCR assays. *Diagn Microbiol Infect Dis* 2012; 73: 111–120.

- 2 Bolt F, Cassiday P, Tondella ML, *et al.* Multilocus sequence typing identifies evidence for recombination and two distinct lineages of *Corynebacterium diphtheriae*. *J Clin Microbiol* 2010; 48: 4177–4185.
- 3 Wagner KS, White JM, Lucenko I, *et al.* Diphtheria in the postepidemic period, Europe, 2000–2009. *Emerg Infect Dis* 2012; 18: 217–225.
- 4 Wilson AP. The return of *Corynebacterium diphtheriae*: the rise of non-toxicogenic strains. *J Hosp Infect* 1995; 30: Suppl. 306–312.
- 5 Funke G, Altwegg M, Frommelt L, *et al.* Emergence of related non toxicogenic *Corynebacterium diphtheriae* biotype mitis strains in Western Europe. *Emerg Infect Dis* 1999; 5: 477–480.
- 6 Belko J, Wessel DL, Malley R. Endocarditis caused by *Corynebacterium diphtheriae*: case report and review of the literature. *Pediatr Infect Dis J* 2000; 19: 159–163.
- 7 Farfour E, Badell E, Zasada A, *et al.* Characterization and comparison of invasive *Corynebacterium diphtheriae* isolates from France and Poland. *J Clin Microbiol* 2012; 50: 173–175.
- 8 Galazka A. The changing epidemiology of diphtheria in the vaccine era. *J Infect Dis* 2000; 181: Suppl. 1, S2–S9.
- 9 Honma Y, Yoshii Y, Watanabe Y, *et al.* A case of afebrile pneumonia caused by non-toxicogenic *Corynebacterium diphtheriae*. *Jpn J Infect Dis* 2009; 62: 327–329.

DOI: 10.1183/09031936.00119612

Influenza vaccine-induced interstitial lung disease

To the Editor:

Influenza is a serious disease that can spread around the world in seasonal epidemics, resulting in the deaths of an estimated 250,000 to 500,000 people every year [1]. Vaccination is the most effective method for preventing secondary complications and the risk of influenza-related hospitalisation and death. The influenza vaccine is safe in general, and the most common side-effects, such as injection-site reaction, pain, fever, myalgia and headache, are not important clinically [2]. However, a few case reports of interstitial lung disease (ILD) caused by influenza vaccine have been published. We report a case of influenza vaccine-induced ILD with a review of the literature.

A 75-yr-old female was referred to our hospital (Komatsu Municipal Hospital, Komatsu, Japan) for evaluation of fever and chest radiograph abnormalities in November 2011. 2 weeks previously she had received the influenza vaccine (trivalent inactivated vaccine: A/California/7/2009 [H1N1]-like, A/Victoria/210/2009 [H3N2]-like, and B/Brisbane/60/2008-like antigens). She had developed a fever 1 week before admission, and a chest radiograph revealed patchy airspace infiltrates in both lungs (fig. 1a). She received garenoxacin without any improvement and was then referred to our hospital for further evaluation. She had a medical history of hypertension, anaemia and chronic renal failure due to diabetes, and had started regular haemodialysis at the age of 74 yrs. She had no past history of pulmonary disease and her chest radiograph the previous month was normal. Her medications included valsartan, furosemide, isosorbide dinitrate and cilnidipine, which had remained unchanged for 2 yrs. She also had an insulin injection every day. She was a nonsmoker and had no allergies to foods or drugs.

On examination her blood pressure was 154/47 mmHg, heart rate was 98 beats·min⁻¹, oxygen saturation was 96% on room air and her temperature was 37.7°C. Physical examination revealed no abnormalities. Chest computed tomography (CT) revealed bilateral ground-glass opacities and patchy infiltration in a predominantly peribronchial and subpleural distribution (fig. 1b). Laboratory findings revealed C-reactive protein of 5.5 mg·L⁻¹ and lactate dehydrogenase level of 539 IU·L⁻¹. Serology tests for several autoimmune markers and tumour markers were negative. The serum levels of Krebs von den

Lungen-6 (KL-6), surfactant protein-D (SP-D) and surfactant protein-A (SP-A) were increased to 1,720 U·mL⁻¹ (normal range <500 U·mL⁻¹), 924 ng·mL⁻¹ (normal range <110 ng·mL⁻¹) and 115 ng·mL⁻¹ (normal range <43.8 ng·mL⁻¹), respectively. Pulmonary function tests were: forced vital capacity (FVC) 1.46 L (normal range 68.2% predicted); forced expiratory volume in 1 s (FEV1) 1.31 L (normal range 95.6% pred); FEV1/FVC ratio 89.7%; and diffusing capacity of the lung for carbon monoxide 5.52 mL·min⁻¹·mmHg⁻¹ (normal range 28.2% pred). There were no abnormalities seen on echocardiography. The findings of the bronchoalveolar lavage (BAL) fluid revealed 45.0% macrophages, 52.7% lymphocytes, 0.3% neutrophils and 2.0% eosinophils. The CD4+/CD8+ ratio of lymphocytes was 1.18. BAL fluid cultures for bacteria, fungi and mycobacteria were negative, and cytology was unremarkable. Transbronchial lung biopsy (TBLB) specimens revealed interstitial inflammation with lymphocytes, mild interstitial fibrosis, and reactive hyperplastic type II pneumocytes. There were no findings of infection, granuloma or malignancy. These findings were consistent with a diagnosis of ILD.

Based on the clinical course, a possible cause was influenza vaccination. The temporal relationship between influenza vaccination and clinical symptoms argued strongly for a causative role of this agent. Several evaluations support the assessment that the ILD could not be explained by other causes, including infections, collagen vascular disease, granulomatous or pulmonary oedema. A drug lymphocyte stimulation test (DLST) on her peripheral lymphocytes gave a positive reaction to the influenza vaccine with a stimulation index of 296% (normal range <180%).

She was started on oral prednisolone at 30 mg daily. Her symptoms and laboratory data were remarkably improved (C-reactive protein 0.1 mg·L⁻¹, lactate dehydrogenase 207 IU·L⁻¹, KL-6 175 U·mL⁻¹, SP-D 17.2 ng·mL⁻¹, SP-A 60.9 ng·mL⁻¹), and the repeated chest radiograph and CT demonstrated almost complete resolution of the bilateral opacities (fig. 1c and d). Prednisolone was then subsequently tapered and was withdrawn at 2 months. She has been observed without recurrence.

Influenza vaccine-induced ILD is rare, and very few studies have focused on the disease. According to the vaccine adverse event reporting system established by the Centers for Disease Control and Prevention and the Food and Drug Administration