Casi clinici

Case reports

Usefulness of (1→3)-β-D glucan in early diagnosing *Pneumocystis jirovecii* pneumonia: a case report

Utilità del (1→3)-β-D glucano nella diagnosi precoce di polmonite da Pneumocystis jirovecii*: un caso clinico*

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INTRODUCTION

Pneumocystis jirovecii pneumoniae (PJP) is a well-known opportunistic infection affecting immunocompromised hosts, especially patients infected with HIV. However, with the rising number of patients receiving immunosuppressive therapy, PJP is being increasingly recognized in immunosuppressed hosts who are not infected with HIV [1]. Invasive diagnostic techniques such as BAL or transbronchial lung biopsy and specific staining of cyst and/or trophozoites are the gold standard for PJP diagnosis [2].

BDG, one of the major components of the cyst wall of *P. jirovecii*, can be a serological marker for the diagnosis and monitoring of PJP; some reports have also supported this evidence [2-4]. (1→3) β-D-glucan (BDG) composes a portion of the cell wall of most fungi, including *Candida*, *Aspergillus*, and other yeasts and moulds such as *Trichosporon, Fusarium, Penicillium, Cephalosporium,* and *Pneumocystis jiroveci* [5]. The serum level of BDG may be useful as a diagnostic and therapeutic indicator [6]. For the early detection of fungal infection, we routinely measure serum BDG and galactomannan levels in immunocompromised patients and start preemptive therapy based on the results. We report an HIV-seronegative patient with acute lymphoblastic leukaemia (ALL) who developed PJP, correlated with increasing serum BDG levels.

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CASE REPORT

A 23-year-old male was admitted to the haematological ward of the Erciyes University Hospital in September 2011 with fever and neutropenia and diagnosed with acute lymphoblastic leukaemia (ALL). The patient was treated with cycles of four Hyper-CVAD/ MTX-Ara-C (cyclophosphamide, vincristine, doxorubicin, and dexamethasone/methotrexate-cytarabine) chemotherapy and achieved complete remission. Two years after the diagnosis, he complained of progressive dyspnea for three months and fever with chills for two days. Cough and sputum were observed. Physical examination revealed a temperature of 38.6 °C, respiratory rate of 25 breaths·min^{-1} and diffuse rales predominantly in the middle and lower chest. On laboratory examination, there was a severe neutropenia (white blood cell count [WBC] 1.07/mm^3 and absolute neutrophil count 430/mm^3). Initial chest film showed bilat-

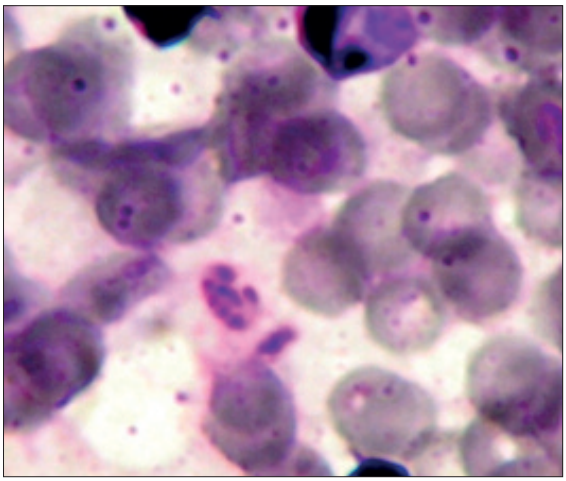

Figure 1 - Giemsa staining (x 1000) of *P. jirovecii* cyst containing up to 8 intracystic bodies.

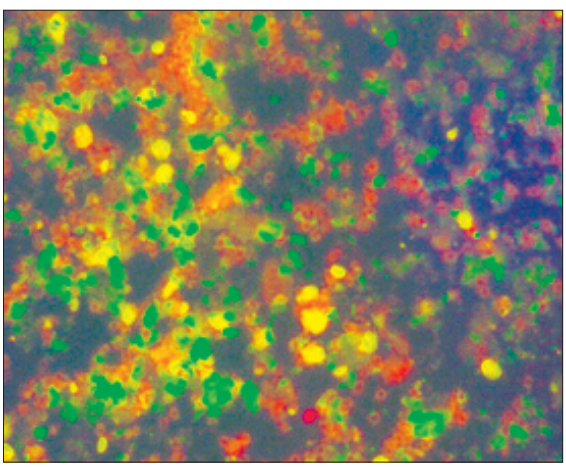

Figure 2 - Immunofluorescence staining (x 400) of *P. jirovecii*, apple-green fluorescence of the round, thick-walled cysts and pleomorphic trophozoites.

eral infiltrates without evidence of pleural effusion. Bone marrow aspirate showed myeloid suppression and no significant increase in blast cells.

He was treated initially with supplemental oxygen and intravenous clarithromycin plus cefepime for seven days, but clarithromycin was changed to levofloxacin because of allergic reaction. However, his condition deteriorated and fever persisted.

The patient received treatment with granulocyte colony stimulating factor (GCSF) until his neutrophil count recovered. Intravenous dexamethasone with aminophylline was administered intermittently to relieve his dyspnea and wheezing. Sputum, blood, and urine cultures were sterile; smears for acid-fast bacilli and tests for viral antibodies were both negative. Serology for HIV was negative. A bronchoscopy with bronchoalveolar lavage was performed, but biopsy could not be carried out because of decreasing oxygen saturation. Mycobacterial and mycologic cultures of BAL were negative. Giemsa and immunofluorescence staining of the BAL detected the existence of *P. jiroveci* in the lungs. The patient was therefore diagnosed with PJP.

For the detection of *P. jirovecii*, a 10-mL BAL fluid was centrifuged at 1,875 *g* for 10 min., and a smear was microscopically examined for the presence of *P. jirovecii* with Giemsa stain and immunofluorescence stain (Cellabs Diagnostics Pty. Ltd. Sydney, Australia) following the recommendations of the manufacturer. Seven days after admission, in Giemsa staining we saw *P. jirovecii* cysts containing up to eight intracystic bodies (Figure 1) and in immunofluorescence staining *P. jirovecii* exhibited bright, apple-green fluorescence of the round, thick-walled cysts and pleomorphic trophozoites in contrast to the reddish-brown colour of counterstained material (Figure 2).

Laboratory values showed rising LDH with a maximum of 621 IU/L (normal 100-245 IU/L). One day and five days after admission, the serum level of BDG was >500 pg/mL (normal range <60 pg/mL) and galactomannan assay in serum was negative.

After detection of *P. jirovecii*, TMP-SMX was added intravenously. The high serum level of BDG was considerably reduced by treatment with TMP-SMX. The fever subsided the following day and the clinical condition of the patient increasingly improved. He was discharged from the hospital and clinical follow up was suggested.

DISCUSSION

We reported herein a case of PJP occurring two years after a patient was diagnosed as having ALL, in which the serum level of BDG was useful as an early diagnostic and therapeutic indicator.

Invasive fungal diseases, as opportunistic infections, are common among haematological malignancy and AIDS patients and account for a growing number of nosocomial infections, particularly among organ transplant recipients and other patients receiving immunosuppressive

treatments [7]. PJP in humans is caused by *P. jirovecii*, which has recently been reclassified as a fungus because its cell wall composition and nucleotide sequences are more similar to those of fungi [8]. PJP may occur in a variety of haematological diseases, including non-Hodgkin's lymphoma, chronic lymphocytic leukaemia, acute leukaemia, chronic myeloid leukaemia, multiple myeloma, myelodysplastic syndrome, Hodgkin's disease and thrombocytopenia [9].

Our patient was diagnosed as having ALL approximately two years before and was admitted to hospital with progressive dyspnea and fever with chills.

Though fever, dry cough and progressive dyspnea with pulmonary infiltrates of an alveolar pattern are typical of PJP, these manifestations are non-specific [10].

PJP may be difficult to diagnose owing to non-specific symptoms and signs, concurrent use of prophylactic drugs, and simultaneous infection with various organisms. Since *Pneumocystis* cannot be cultured, the diagnosis of PJP requires microscopic examination in order to identify *P. jirovecii* from a clinically relevant source such as specimens of induced sputum, BAL fluid or lung tissue [11]. BAL has traditionally been the definitive clinical sample. However, the procedure to obtain the specimen is invasive while the induced sputum sample is not sensitive compared to BAL and lung tissue for detection of *P. jiroveci* [12].

We detected the existence of *P. jiroveci* in the lung by Giemsa and immunofluorescence staining of the BAL. With Giemsa stain *P jirovecii* cysts are variably stained and intracystic bodies are present in different numbers within the cysts. Mature cysts have eight intracystic bodies. Some cysts appear empty. Serum testing for the diagnosis of PJP has not yet been established. Although an elevated serum LDH level has been noted in patients with PJP, it is likely to be a reflection of the underlying lung inflammation and injury rather than a specific marker for the disease. [13].

On the other hand, Obayashi et al. reported that, serum levels of LDH and BDG were significantly higher in PJP-positive patients. In our patient, one day after admission, both serum levels of LDH and BDG were significantly higher. As *P. jirovecii* is more closely related to fungi than to protozoa, the measurement of plasma BDG, which is a cell wall constituent of fungi, may be useful for both the diagnosis and the therapeutic monitoring of PCP [14]. The BDG assay we used (Fungitell; Associates of Cape Cod) is made from *Limulus polyphemus* found on the Atlantic coast of North America between Florida and the Gulf of Maine.

Certain fungal species produce very low levels of BDG and are not detected by the Fungitell assay. These include the genus *Cryptococcus* as well as *Zygomycetes* such as *Absidia*, *Mucor* and *Rhizopus* [15]. Persat et al. reported that the BDG test would be the first valuable non-invasive serodiagnostic tool for *P. jiroveci* pneumonia and would be particularly useful for patients for whom bronchoscopy was sometimes problematic [3].

Compared with infection caused by other pathogens, PJP can be significantly overlooked, especially in the presence of neutropenia. Our case developed PJP in the setting of febrile neutropenia.

The patient received broad-spectrum antibiotics as the initial empirical treatment.

In conclusion, PJP should be considered in the early phase of febrile neutropenia in patients with haematological malignancies. We experienced a case of PJP that was diagnosed by Giemsa and immunofluorescence stains in BAL; also, the patient's serum level of BDG was useful as diagnostic and therapeutic indicator. We suggest that measuring BDG could be considered as a primary modality for early diagnosis of PJP, especially for patients in whom bronchoscopy is problematic because of respiratory status, or for those with negative sputum or bronchoscopic lavage sampling results for whom the clinical suspicion for PJP remains high. Future studies should explore the relationship of this marker to clinical outcomes and prognosis.

Keywords: Pneumocystis jirovecii pneumoniae, acute lymphoblastic leukaemia, (1 3)- -D-glucan.

SUMMARY

Pneumocystis jirovecii pneumoniae (PJP) may be difficult to diagnose. Since pneumocystis cannot be cultured, the diagnosis of PJP requires microscopic examination to identify pneumocystis from induced sputum or bronchoalveolar lavage (BAL) fluid. In order to evaluate the usefulness of (1 3) ß-D-glucan (BDG) levels in the early diagnosis of PJP, we describe the case of PJP in a 25-year-old male with acute lymphoblastic leukaemia (ALL) admitted to hospital with progressive dyspnea and fever with chills. The patient was not infected with human immunodeficiency virus (HIV). Sputum, blood, and urine cultures were negative; smears for acid-fast bacilli and tests for viral antibodies were both negative. The microbiology study of the BAL with Giemsa and immunofluorescence staining, seven days after admission showed the existence of *P. jiroveci* in the lungs. Further, one day and five days after admission, (1 3) ß-D-glucan (BDG) levels were very high. The high serum level of BDG considerably decreased after treatment with trimethoprim-sulfamethoxazole (TMP-SMX) and the clinical condition of the patient increasingly improved.

RIASSUNTO

La polmonite da Pneumocystis jirovecii (PJP) può porre delle difficoltà diagnostiche. Infatti, in assenza di tecniche colturali per pneumocystis, la diagnosi di PJP richiede l'esame microscopico per l'identificazione di pneumocystis dall'espettorato indotto o dal liquido di lavaggio broncoalveolare (BAL). Al fine di valutare l'utilità dei livelli di (1 3) ß-D-glucano (BDG) nella diagnosi precoce di PJP, nel presente articolo descriviamo un caso di PJP occorso in un uomo di 25 anni affetto da leucemia linfoblastica acuta (LLA) ricoverato in ospedale con dispnea progressiva e febbre con brividi. Il paziente non era affetto da HIV. Gli esami colturali dell'espettorato, del sangue e delle urine erano negativi; gli strisci per la ricerca di bacilli acido-alcol resistenti e i test per la ricerca di anticorpi virali erano entrambi negativi. L'esame microbiologico del BAL con colorazione di Giemsa e immunofluorescenza, condotto dopo sette giorni dal ricovero metteva in luce la presenza di P. jiroveci nei polmoni. Inoltre, dopo uno e cinque giorni dal ricovero i livelli di (1 3) ß-D-glucano (BDG) erano molto elevati. L'alto livello sierico di BDG si è poi considerevolmente ridotto a seguito di trattamento con trimetoprim/sulfametossazolo (TMP-SMX) e le condizioni del paziente sono progressivamente migliorate.

REFERENCES

[1] Cheng V.C., Hung I.F., Wu A.K., Tang B.S., Chu C.M., Yuen K.Y. Lymphocyte surge as a marker for immunorestitution disease due to *Pneumocystis jirovecii* pneumonia in HIV-negative immunosuppressed hosts. *Eur. J. Clin. Microbiol. Infect. Dis* 23, 512-514, 2004.

[2] Nakamura H., Tateyama M., Tasato D., et al. Clinical utility of serum β-d-glucan and KL-6 levels in *Pneumocystis jirovecii* pneumonia. *Intern. Med.* 48, 195-202, 2009.

[3] Persat F., Ranque S., Derouin F., Michel-Nquyen A., Picot S., Sulahian A. Contribution of the (1→3)-β-D-glucan assay for diagnosis of invasive fungal infections. *J. Clin. Microbiol.* 46, 1009-1013, 2008.

[4] Shimizu A., Oka H., Matsuda T., Ozaki S. (1→3) β-D glucan is a diagnostic and negative prognostic marker for *Pneumocystis carinii* pneumonia in patients with connective tissue disease. *Clin. Exp. Rheumatol.* 23, 678-680, 2005.

[5] Akamatsu N., Sugawara Y., Kaneko J., Tamura S., Makuuchi M. Preemptive treatment of fungal infection based on plasma (1→3) β-D-glucan levels after liver transplantation. *Infection* 35, 346-351, 2007.

[6] Kawagishi N., Miyagi S., Satoh K., Akamatsu Y., Sekiguchi S., Satomi S. Usefulness of beta-D glucan in diagnosing *Pneumocystis carinii* pneumonia and monitoring its treatment in a living-donor liver-transplant recipient. *J. Hepatobiliary Pancreat. Surg.* 14, 308-311, 2007.

[7] Alexander B. Diagnosis of fungal infection: new technologies for the mycology laboratory. *Transpl. Infectious Dis.* 4 (Suppl. 3), 32-37, 2002.

[8] Lu J.J., Lee C.H. Pneumocystis pneumonia. *J. Formos Med. Assoc.* 107, 830-842, 2008.

[9] Chang H., Yeh H.C., Su Y.C., Lee M.H. *Pneumocystis jiroveci* pneumonia in patients with non-Hodgkin's lymphoma receiving chemotherapy containing rituximab. *J. Chin. Med. Assoc.* 71, 579-582, 2008.

[10] Thomas C.F., Limper A.H. Pneumocystis pneumonia. *N. Engl. J. Med.* 350, 2487-2498, 2004.

[11] Sadatomo Tasaka S., Hasegaw H., Kobayashi S., et al. Serum indicators for the diagnosis of pneumocystis pneumonia. *Chest* 131, 1173-1180, 2007.

[12] Pinlaor S., Mootsikapun P., Pinlaor P., et al. PCR diagnosis of *Pneumocystis carinii* on sputum and bronchoalveolar lavage samples in immuno-compromised patients. *Parasitol. Res.* 94, 213-218, 2004.
[13] Quist J., Hill A.R. Serum lactate dehydrogenase in *Pneumocystis carinii* pneumonia, tuberculosis, and bacterial pneumonia. *Chest* 108, 415-418, 1995.
[14] Obayashi T., Yoshida M., Mori T., et al. Plasma (1→3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 345, 17-20, 1995.
[15] Odabasi Z., Paetznick V.L., Rodriguez J.R., Chen E., McGinnis M.R., Ostrosky-Zeichner L. Differences in beta-glucan levels of culture supernatants of a variety of fungi. *Med. Mycol.* 44, 267-272, 2006.