

Allergic Bronchopulmonary Mycosis due to Exposure to *Eurotium herbariorum* after the Great East Japan Earthquake

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Abstract

Background: Indoor mold levels typically increase after natural disasters, flooding, and water damage. *Eurotium herbariorum* is the sexual stage of *Aspergillus glaucus*.

Case Presentation: A 66-year-old, Japanese male, ex-smoker had been diagnosed with bronchial asthma when he was five years old; he achieved remission at the age of 13 years. He was displaced from his home during the Great East Japan Earthquake on March 11, 2011 and moved to temporary housing in Miyagi Prefecture in June 2011. He experienced the first episode of chest tightness, coughing, and wheezing in February 2012, when he again was diagnosed as having bronchial asthma. Mycofloral surveillance detected high counts of *Eurotium* in the air of his bedroom, kitchen, and living room, with a maximal fungal count of 163,200 colony-forming units per cubic meter (CFU/m³). Although *Cladosporium* and *Penicillium* typically predominate in the indoor air of residential dwellings, only low levels of these organisms were present in the patient's home. Morphologic identification confirmed the isolates as *E. herbariorum*. The patient had positive reactions to *E. herbariorum* in skin prick testing and the presence of antigen-specific precipitating antibodies to *E. herbariorum*. Computed tomography of the chest in August 2013 revealed central bronchiectasis and bronchial wall thickening. The patient experienced late reactions after provocation testing with *E. herbariorum*.

Conclusion: This report presents the rare case of a patient who developed allergic bronchopulmonary mycosis (ABPM) due to exposure to *E. herbariorum* during temporary housing after the Great East Japan Earthquake.

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Introduction

Indoor mold counts typically increase after flooding and water damage,¹ and cases of respiratory diseases usually increase after earthquakes.² The most common cause of allergic bronchopulmonary mycosis (ABPM) is *Aspergillus fumigatus*. *Aspergilli* other than *A. fumigatus* that have been reported to cause ABPM include *A. niger*, *A. sydowii*, and *A. terreus*.³ *Eurotium herbariorum* is the name usually given to the sexual stage of *A. glaucus*. Exposure of children living on farms to *Eurotium* species decreases their risk of developing atopy and asthma.⁴ In contrast, increases in *A. versicolor* and *Eurotium* counts in school buildings with moisture damage have been associated with increased prevalence of asthma.⁵ *Eurotium herbariorum* has not previously been reported to cause ABPM.

Abbreviations:

ABPM: allergic bronchopulmonary mycosis
CFU: colony-forming units per cubic meter
DG18: dichloran – glycerol agar
DRBC: dichloran – rose bengal – chloramphenicol agar
FEV1: forced expiratory volume in one second

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Case Presentation

A 66-year-old, Japanese male, ex-smoker (packs per year: 75.25) was initially diagnosed with bronchial asthma at the age of five years, and he achieved remission at the age of 13 years. He was displaced from his home during the Great East Japan Earthquake on March 11, 2011 and moved to temporary housing in Miyagi Prefecture in June 2011 at the age of 62 years. He experienced the first episode of chest tightness, coughing, and wheezing in February 2012, and he was diagnosed as having bronchial asthma in March 2012. He continued to experience asthma exacerbations, even after the initiation of fluticasone (100 µg) plus salmeterol (50 µg) twice daily; notably, he experienced no symptoms during a 10-day absence from the temporary housing.

From June 2013, time-course surveillance was performed of fungal mycoflora in the temporary housing. Fungal cells per 10L of air were sampled on a single agar plate by using an Air IDEAL 3P air-sampling system (bioMérieux; Marcy l'Etoile, France) placed approximately one meter above floor level. Air samples were collected at each sampling point of the bedroom, living room, kitchen, and outdoors for a total of 10 culture plates, including five plates of dichloran – rose bengal – chloramphenicol agar (DRBC; Thermo Fisher Scientific; Baintree, Massachusetts USA) and five plates of dichloran – glycerol agar (DG18; Thermo Fisher Scientific). Total fungal counts were obtained; in addition, colonies belonging to the genera *Aspergillus*, *Cladosporium*, *Penicillium*, or *Eurotium* were counted separately by each genus according to the macroscopic and microscopic features of the colonies. Total and genus-specific fungal counts were expressed as the number of colony-forming units per cubic meter (CFU/m³), as estimated by using the most-probable-number method.⁶

The initial round of mycofloral surveillance detected high counts of *Eurotium* in the air of all three indoor areas sampled (the bedroom, kitchen, and living room), with a maximal fungal count of 163,200 CFU/m³ compared with fungal counts of no more than 1000 CFU/m³ in normal housing (Figure 1). This result was noteworthy because *Cladosporium* and *Penicillium* fungi typically predominate in the indoor air of normal dwellings, including the homes of allergic patients.⁷ In view of these results, the patient was instructed on the use of allergen avoidance techniques. Molecular methods were applied to confirm the species-level identification of isolates of *E. herbariorum*. Partial nucleotide sequences of the β-tubulin gene were determined as previously described.⁸ The nucleotide sequences that were obtained here were used as query sequences in a BLAST search of sequences previously registered in GenBank (National Center for Biotechnology Information, National Institutes of Health; Bethesda, Maryland USA). The results of the BLAST were consistent with those from morphologic identification and confirmed that the organism was *E. herbariorum*.

Written informed consent was obtained from the patient to perform skin prick tests against *A. fumigatus* and *E. herbariorum*. The wheal and flare sizes were evaluated at 15 minutes after *A. fumigatus* or *E. herbariorum* pricked compared with that of wheal size after histamine done. He confirmed positive reactions to both *A. fumigatus* (20 x 20 mm; Torii Pharmaceutical; Tokyo, Japan) and *E. herbariorum* (18 x 16 mm; equivalent to *A. glaucus*; the antigen used was a pure culture of an isolate from his temporary housing) compared with 12 x 10 mm after pricked histamine. In addition, the total serum IgE level and presence of antigen-specific IgE antibodies were evaluated by ELISA; the total IgE level was 112.0 IU/mL, and antigen-specific IgE

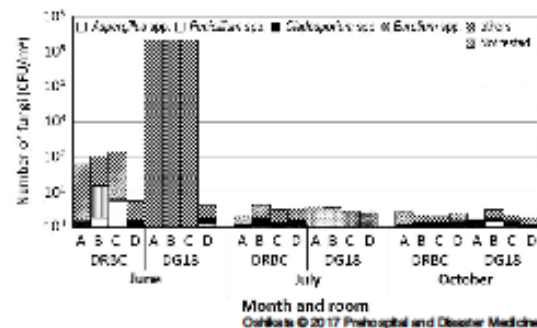


Figure 1. Time Course of Surveillance of Fungal Mycoflora in the Patient's Temporary Housing Before and After the Incorporation of Allergen Avoidance Techniques; A: Living Room; B: Bedroom; C: Kitchen; D: Outdoors.

Note: In June, *Eurotium* predominated and completely covered the plates, and other genera could not be counted. In July and October, *Eurotium* colonies were included in the counts for *Aspergillus*.

Abbreviations: CFU, colony-forming units per cubic meter; DG18, dichloran – glycerol agar; DRBC, dichloran – rose bengal – chloramphenicol agar.

antibodies against *A. fumigatus* but not *A. glaucus* were detected. Ouchterlony double-immunodiffusion testing⁹ of the patient's serum confirmed the presence of antigen-specific precipitating antibodies to *A. glaucus* (Greer; Lenoir, North Carolina USA) and *E. herbariorum*, but not to *A. fumigatus* (Torii Pharmaceutical). Double diffusion between *A. glaucus* and *E. herbariorum* was confirmed by the Ouchterlony assay, which suggested the presence of common antigenicity between them. Central bronchiectasis in right B3 and in left B2 (arrows) was present more clearly in April 2014 than in August 2013 (Figure 2), and greater bronchial wall thickening was seen in April 2014 than in August 2013. He did not have any other known causes of bronchiectasis.

After obtaining written informed consent from the patient, he was performed bronchial provocation tests with *A. fumigatus* and *E. herbariorum*. Ten minutes after antigen-specific provocation with *A. fumigatus* or *E. herbariorum* at 10 mg/mL, the patient developed wheezing (*E. herbariorum*) and chest tightness (*A. fumigatus*), and his forced expiratory volume in one second (FEV1) decreased by 18.7% (FEV1: 1.71 L/s to 1.39 L/s; *A. fumigatus*) or 14.4% (FEV1: 1.87 L/s to 1.60 L/s; *E. herbariorum*) of that before provocation. In addition, he experienced late reactions, with symptoms such as wheezing or chest tightness and the decrease of peak expiratory flow at seven hours (*E. herbariorum*) to 20 hours (*A. fumigatus*) after the last antigen provocation.

The diagnosis of this patient was consistent with ABPM given his positive skin tests to both *E. herbariorum* and *A. fumigatus*, the presence of antigen-specific precipitating antibodies to *A. glaucus* and *E. herbariorum*, and central bronchiectasis with bronchial wall thickening. He did not have any other known causes of bronchiectasis. This patient experienced the first episode of chest tightness, coughing, and wheezing in February 2012. The case was unusual because the duration for sensitization to onset was so short (ie, only eight months). It is possible that this patient had a low total IgE level owing to the short duration of exposure to

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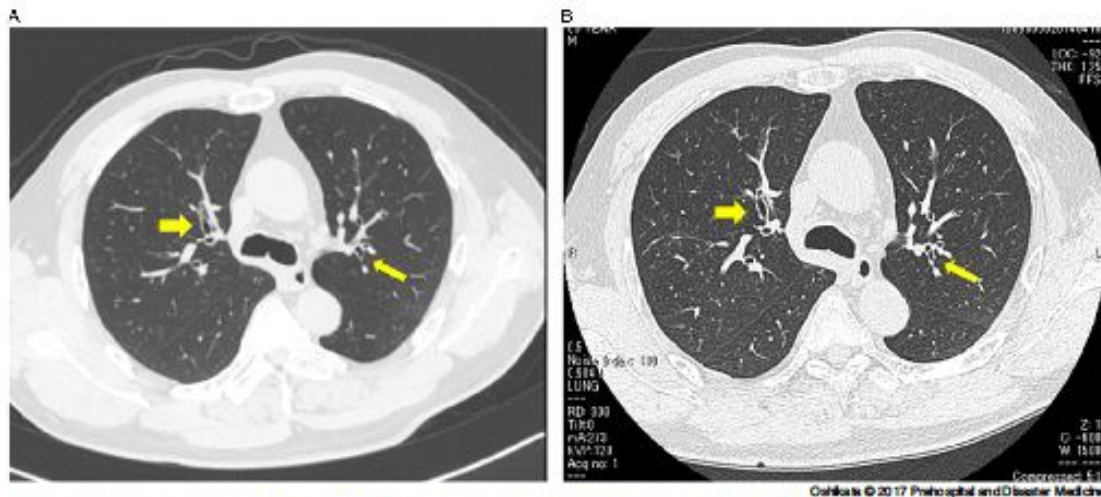


Figure 2. Computed Tomography Scan of the Upper Lungs of Patient, Performed in A: August 2013 and B: April 2014. Note: Central bronchiectasis in right B3 and in left B2 (arrows) was more prominent in April 2014 than in August 2013, and bronchial wall thickness was greater in April 2014 than in August 2013.

E. herbariorum before his ABPM illness. From the data obtained, he was diagnosed as having ABPM caused by *E. herbariorum* during temporary housing after the Great East Japan Earthquake.

The patient's twice-daily fluticasone dose was increased from 100 to 250 μg ; he continued to receive salmeterol (50 μg) twice daily, and 10 mg of a leukotriene antagonist was added to his treatment regimen. The successful avoidance of exposure for *E. herbariorum* might be able to be treated without receiving systemic corticosteroids. In addition, all tatami was removed from his living quarters. The tatami mat is made from rushes and is to be laid on the floor in the room.

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Total and *E. herbariorum*-specific fungal counts obtained one and four months after these allergen avoidance measures had been initiated were markedly lower than the initial counts (Figure 1). He did not have further exacerbations in temporary housing over the four months after the avoidance of allergens.

Conclusion

This report presents the rare case of a patient who developed ABPM due to exposure to *E. herbariorum* during temporary housing after the Great East Japan Earthquake.

Allergic bronchopulmonary mycosis caused by *Penicillium luteum*Chiyako Oshikata^{a,b}, Maiko Watanabe^c, Akemi Saito^d, Hiroshi Yasueda^d, Kazuo Akiyama^b, Yoichi Kamata^c, Naomi Tsurikisawa^{a,b,*}^a National Hospital Organization Saitama National Hospital, Department of Respiratory, 2-1 Sowa, Wako, Saitama 351-0102, Japan^b National Hospital Organization Sagami National Hospital, Department of Allergy and Respiratory, Sakuradai 18-1, Minami-ku, Sagami, Kanagawa 252-0392, Japan^c Division of Microbiology, National Institute of Health Science, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan^d National Hospital Organization, Sagami National Hospital, Clinical Research Center for Allergy and Rheumatology, 18-1 Sakuradai, Minami-ku, Sagami, Kanagawa 252-0392, Japan^{*} Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan

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ABSTRACT

A 65-year-old Japanese male had severe bronchial asthma had increased mold-containing sputum. Serum total IgE level had increased to 798 IU/mL and antigen-specific precipitating antibodies to *P. luteum* and *P. notatum* were present but not those reactive toward any species of *Aspergillus*. Chest computed tomography revealed central bronchiectasis and bronchial wall thickness. After antigen-specific provocation with 10 mg/mL of *P. luteum*, the patient developed asthma exacerbation, but not with *A. fumigatus*. We present a rare case of *Penicillium*-induced allergic bronchopulmonary mycosis caused by *P. luteum*.

1. Introduction

Aspergillus fumigatus is the most common cause of Allergic bronchopulmonary mycosis (ABPM). However, even though *Penicillium* species are among the most common fungi in the environment, ABPM due to *Penicillium* species is rare, accounting for only 1.9% of ABPM cases other than *Aspergillus fumigatus* [1]. In particular, only one case of *Penicillium*-associated ABPM for which the species was identified (*P. digitatum* or *P. rubrum*) has been reported [2]. Here, we present a rare case of *Penicillium*-associated ABPM, which was caused by *P. luteum*.

2. Case

A 65-year-old Japanese male ex-smoker (Pack-year; 58.5) reported having symptoms consistent with severe asthma since he was 36 years old. He had low lung function (forced expiratory volume in 1 s [% FEV₁], 34.2%) and severe hyperresponsiveness to acetylcholine. The provocative acetylcholine concentration that yielded a 20% decrease in FEV₁ was 0.197 mg/mL. In addition, he mounted immediate positive cutaneous reactions to mites, *Trichophyton* spp., and six different pollens, but not to *Aspergillus* or *Penicillium*. He required treatment for several asthma exacerbations annually despite receiving 900 µg of inhaled chlorofluorocarbon – beclomethasone dipropionate and 10 mg

of oral prednisolone daily.

When the patient was 54 years old, resection of nasal polyps followed by daily treatment with 1600 µg inhaled fluticasone propionate and 5 mg of prednisolone decreased the number of asthma exacerbations annually. At age 64 years (day 0), the patient had increased mold-containing sputum, the percentage of eosinophils as 13.8% (cells) and his serum total IgE level had increased from 259 IU/mL at age 39 years to 798 IU/mL. At this time (day 28), we again measured antigen-specific serum IgE levels as described and serum antigen-specific precipitating antibodies by Ouchterlony double immunodiffusion testing. Antigen of *P. chrysogenum* for measurement of IgE or *P. luteum* for precipitating antibodies was derived from Torii Pharmaceutical Co., Ltd, Tokyo, Japan. In contrast to his earlier results, the patient now had antigen-specific IgE antibodies to *Aspergillus* and *Penicillium*. In addition, antigen-specific precipitating antibodies to *P. luteum* and *P. notatum* were present but not those reactive toward any of 9 species of *Aspergillus*. *Penicillium* species was separated from mold-containing sputum at age 64 years, but more detailed species was not identified. Chest computed tomography revealed bronchial wall thickness, central bronchiectasis, and mucoid impaction (Fig. 1) (day 84).

We obtained written informed consent from the patient to perform bronchial provocation tests using *P. luteum* and *A. fumigatus*. At bronchial provocation testing using *Penicillium* 110 min after antigen-

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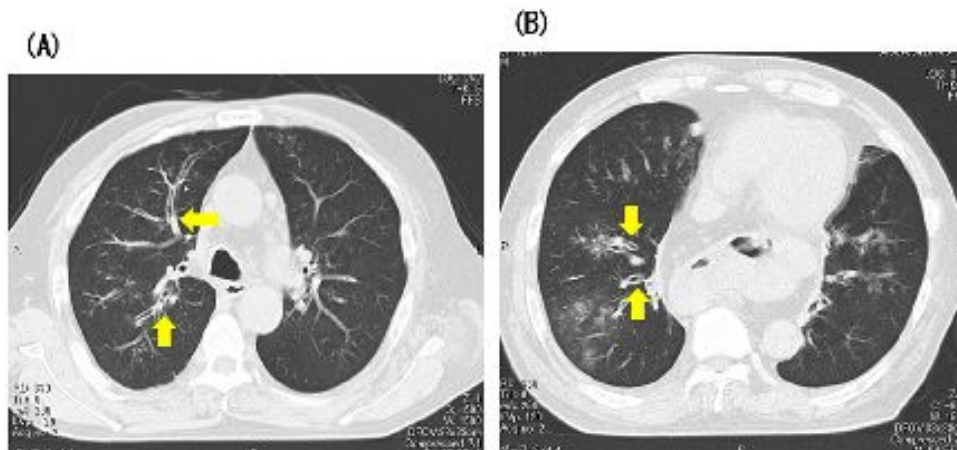


Fig. 1. Computed tomography of the lung, performed at diagnosis. Computed tomography of the upper lung (A), and lower lung (B). Central bronchiectasis was present in right B3, B2 (A), and right B8, B9 (B) and B10 (arrows). Mucoid impaction was shown in right B3 (A) (arrows). Bronchial wall thickness was shown in right B8, B9 (B) and B10 (arrows).

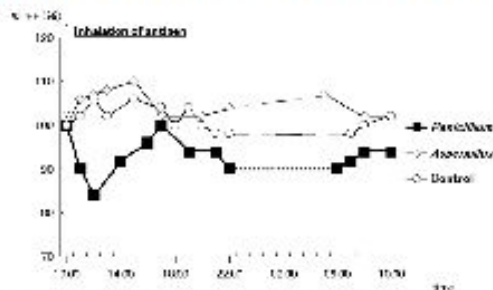


Fig. 2. Results of bronchial provocation testing using *Penicillium luteum* or *Aspergillus fumigatus*. The patient was exposed to the indicated doses of *P. luteum* (solid squares) or *A. fumigatus* (open diamonds) or a negative control antigen (open circles), and the change in peak expiratory flow (%PEF) over in 1 min from the baseline value (100%) was recorded. A decrease of more than 15% (infimal line) from baseline was defined as a positive reaction to the provocation protein fraction.

specific provocation with 10 mg/mL of *P. luteum* (Torii Pharmaceutical Co., Ltd, Tokyo, Japan), the patient developed wheezing and chest tightness, and his peak expiratory flow decreased to 83.5% of that before antigen administration (day 98). He experienced a delayed hypersensitivity reaction 12 h after last provocation (day 99). However, inhalation of *A. fumigatus* (Torii Pharmaceutical Co., Ltd., Tokyo, Japan) did not elicit any changes in lung function or cause asthma exacerbation (Fig. 2) (day 105).

To collect particulates aerosolized from the patient's room or environment (day 133), we left open Petri dishes (plain coated with potato dextrose agar) plastic, 90×15 mm; SH90-15; Asahi Glass Co. Ltd., Tokyo, Japan) throughout the patient's bedroom for 10 min according to a report by Takatori et al. [3]. In addition, to collect airborne particulate matter, we distributed pieces of adhesive tape (Tegaderm Transparent Dressing 1625WJ; 6×7 cm; 3M Health Care, St Paul, MN) throughout his living spaces. And these samples by patient's sputum were collected. These samples were cultured at 25 or 37 °C for several days; resulting colonies were identified by using morphologic evaluation and molecular methods. Specifically, partial sequences of the β -tubulin gene obtained by using the primers Bt2a and Bt2b were subjected to BLAST analysis.

To identify the antigen from the sputum for cultures, we added 1.5 mL of glass beads (Biospec Products, Bartlesville, OK) and homogenized the mixture by using a Mini-Beadbeater (Biospec Products). It

was then incubated with 0.125 M NH_4CO_3 overnight at 4 °C; the antigen was extracted after freeze-drying of the filtrate. Samples cultured from the patient's bedroom, balcony, and parents' house contained multiple species of *Penicillium*, but not *P. luteum* (Table 1). However, the patient's asthma symptoms decreased after a healthy potted pothos plant in his living room was transplanted from mud into water (day 224); the mud from the cultivated pothos contained numerous colonies of diverse *Penicillium* species. In view of the collected data and observations, we diagnosed ABPM due to *P. luteum*.

3. Discussion

The genus *Penicillium* was first described more than 200 years ago [4], and since then, more than 1000 organisms have been assigned to it. A phylogenetic species concept has been used for *Penicillium* classification and identification and was facilitated by the incorporation of DNA sequencing techniques in the 1990s [5]. Both *Aspergillus* and *Penicillium* fungi typically are found indoors and are among the genera most frequently encountered in these environments. In addition, the high frequencies of identical residues and conservative substitutions between the amino acid sequences of *Aspergillus* and *Penicillium* [6] have led to inaccuracies and confusion regarding the taxonomy of these fungi. We did not examine precipitating antibodies to other *Penicillium* species than *P. luteum* or *P. notatum*. Furthermore, we performed bronchial provocation tests using *P. luteum*, but not *P. notatum*, and any other *Penicillium* species than *P. luteum*. Because we could not get commercially available antigens or not purified from environment or sputum. We considered the possibility for crossreactivity in *P. luteum* or *P. notatum* or other *Penicillium* species. We could not find *P. luteum* in his sputum or his living spaces. We consider *P. luteum* may be mixed in *Penicillium* species. Many kind species of *Penicillium* were resembling similar and it was difficult to identify each *Penicillium*. However, this patient exacerbated asthma after provocation of *P. luteum* but not *A. fumigatus*. We consider one of some causes for ABPM in this patient is due to *P. luteum*.

We elucidate a crossreactivity of *P. luteum* and *Penicillium* species in the future.

Here, we presented a rare case of *Penicillium*-specific ABPM, which was due to *P. luteum* and was not associated with *Aspergillus*, the most common cause of the disease. Despite our inability to isolate *P. luteum* from our patient's living spaces, we surmised that it was present among the *Penicillium* spp. in the soil of a potted pothos plant. Alternatively, the patient may have harbored antigen-specific antibodies for species

Table 1
Molecular identification of *Penicillium* species from patient's sputum and living environment.

	<i>P. brevisporium</i>	<i>P. citrinigrum</i>	<i>P. citrinum</i>	<i>P. gubatum</i>	<i>P. isabellum</i>	<i>P. latium</i>	<i>P. stipitissimum</i>	<i>P. strobil</i>	<i>Penicillium</i> spp.	<i>Talaromyces</i> spp.
Sputum								++		
Environment										
Backroom										
Living room			++							
soil of pebbles plant			++							
Dining room			+							
Balcony			+							
soil of Benjamina plant			+							
soil of rosemary plant			+							
Percent house	+							++		

+, present in one sample only; ++, present in 2 or more samples.

closely related to *P. latium* that may cross-react with *P. latium* antigen. In this study, we isolated several strains (noted as "*Penicillium* spp." in Table 1) that are morphologically or molecularly similar to *P. latium*. The patient's sensitization to *Penicillium* but not *Aspergillus* may reflect his exposure to higher counts of, and more, *Penicillium* species compared with *Aspergillus* species.

Conflict of interest

The authors report no conflicts of interest in this work.

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