

## GENOTYPING SUPPORTS THE CLINICAL SIGNIFICANCE OF ISOLATION OF *ASPERGILLUS FUMIGATUS* FROM SPUTUM AND BRONCHIAL SECRETION SAMPLES FOR THE DIAGNOSIS OF INVASIVE PULMONARY ASPERGILLOSIS

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**Objectives:** In the absence of histopathology of lung biopsies, the bronchoalveolar lavage (BAL) sample is preferred for the diagnosis of invasive pulmonary aspergillosis (IPA), although collection is commonly contraindicated. Isolation of *Aspergillus fumigatus* from sputum and bronchial secretion samples is commonly interpreted as colonisation or laboratory contamination, particularly in non-neutropenic patients. By means of genotyping, we showed that isolation of *A. fumigatus* from sputum/bronchial secretions from patients fulfilling the criteria for IPA should be interpreted as significant.

**Methods:** We studied 13 patients with simultaneous ( $\pm 33$  days) isolation of *A. fumigatus* in BAL samples and sputum/bronchial secretion samples from 2006 to 2012. Patients were classified as having proven IPA (n=1) or probable IPA (n=12) according to the revised criteria of the European Organisation for Research and Treatment of Cancer; patients with chronic obstructive pulmonary disease fulfilled Bulpa's criteria. Haematological cancer (23%) and chronic obstructive pulmonary disease (46%) were the most frequent underlying conditions (Table). All colonies (n=139) found in the BAL (n=13), sputum (n=21), and bronchial aspirate (n=6) cultures were studied. Isolates were identified by sequencing the  $\beta$ -tubulin gene and genotyped using STRaf. Considering the genotypes found in the BAL samples as causative of IPA, genotypes from BAL samples and sputum/bronchial secretion samples were identical when they showed the same alleles for all markers.

**Results:** We found 2 or more genotypes in 3/21 sputum samples (14%), in 3/13 BAL samples (23%), and in 3/6 bronchial secretion samples (50%). The number of genotypes per sample type ranged from 1 to 3 in BAL samples and from 1 to 6 in sputum/bronchial secretion samples. The number of genotypes per patient ranged from 1 to 6. In 12 of the 13 patients (92%), we found matching genotypes in both the BAL and the sputum/bronchial secretion samples (Table).

**Conclusion:** We found matching genotypes in both the BAL samples and the sputum/bronchial secretion samples in 92% of the patients studied, suggesting that the genotyping pattern found in samples obtained non-invasively mirrors that of the genotypes causing the infection. Our observations support the clinical significance of isolation of *A. fumigatus* in samples of sputum or bronchial secretion from patients with clinical suspicion of invasive aspergillosis.

Patient	Underlying conditions	Radiology	GM serum/BAL (>0.5)	No. of colonies studied/ genotypes found		BAL and sputum/bronchial secretion matches	Diagnosis
				BAL	Sputum/bronchial secretions		
3161	COPD	Bilateral multifocal consolidation, necrosis	+ / Not done	1/1	11/2	Yes	Probable IPA
3439	Haematological cancer	Nodules Infiltrates	+ / +	6/1	3/1	Yes	Proven IPA
3446	COPD, solid cancer	Bilateral infiltrates	+ / Not done	6/4	13/3	Yes	Probable IPA
3581	HIV	Reticulo-nodular infiltrates	+ / +	7/1	6/1	Yes	Probable IPA
3625	Liver disease	Bilateral and multifocal consolidated cavitations	+ / +	6/2	2/1	Yes	Probable IPA
3654	HIV, liver disease	Bilateral infiltrates	+ / +	6/1	5/1	Yes	Probable IPA
3699	COPD, solid cancer	Cavitations	+ / +	4/1	2/1	Yes	Probable IPA
3703	COPD, solid cancer	Nodules, cavitations	+ / +	1/1	4/1	Yes	Probable IPA
3818	Haematological cancer, COPD	Bilateral infiltrates	+ / +	7/1	13/1	Yes	Probable IPA
3899	Solid cancer	Bilateral infiltrates, cavitations	+ / +	2/2	1/1	Yes	Probable IPA
3950	Corticosteroids	Bilateral infiltrates	+ / +	2/1	12/6	Yes	Probable IPA
3559	COPD	Infiltrates	- / -	7/1	3/3	Yes	Probable semi-IPA
3965	Haematological cancer	Nodules, cavitations	- / Not done	2/1	2/2	No	Probable IPA