Microsatellite (STRAf) Genotyping Cannot Differentiate between Invasive and Colonizing Aspergillus fumigatus Isolates

Pilar Escribano, a,b,c Teresa Peláez, a,b,c,d Emilio Bouza, a,b,c,d Jesús Guinea a,b,c,d

Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Universidad Complutense de Madrid, Madrid, Spain; Instituto de Investigación Sanitaria del Hospital Gregorio Marañón, Madrid, Spain; CIBER de Enfermedades Respiratorias (CIBERES CB06/06/0058), Madrid, Spain; Medicine Department, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain

We studied whether short tandem repeats of Aspergillus fumigatus (STRAf) can differentiate between invasive and colonizing genotypes of A. fumigatus. Of the 395 genotypes detected \((n = 1,373)\) isolates, 50 were clusters and 24 \((6\%)\) of all genotypes involved the patients with invasive aspergillosis and those colonized with A. fumigatus, indicating that genotyping cannot discriminate between invasive and colonizing isolates.

Diagnostics invasive aspergillosis is challenging and is based on host factors, radiological findings, and mycological criteria \(1\). Culture of Aspergillus species is widely used, although it is impaired by low specificity in some groups of patients, as shown by the high proportion of nonhematological patients with Aspergillus isolated from lower respiratory tract samples but no invasive disease \(2, 3\). Therefore, the isolation of Aspergillus fumigatus should be interpreted with caution.

A. fumigatus is a ubiquitous fungus that is able to colonize or infect patients depending on the integrity of the immune system \(4\); however, different degrees of virulence have been observed in clinical isolates of A. fumigatus \(5, 6\). Hypothetically, some isolates cause invasive aspergillosis, whereas others only colonize patients. Unfortunately, to our knowledge, the association between specific isolates and the presence of invasive aspergillosis has not been studied.

This study was presented in part at the 24th European Congress on Clinical Microbiology and Infectious Diseases, Barcelona, Spain, 10 to 13 May 2014.

A large collection of clinical A. fumigatus sensu stricto isolates was analyzed to determine whether microsatellite (short tandem repeats of Aspergillus fumigatus [STRAf]) genotyping was able to differentiate between isolates from patients with invasive aspergillosis and isolates from colonized patients.

A total of 95 patients admitted to Hospital General Universitario Gregorio Marañón between 2005 and 2012 and diagnosed with proven \((n = 16)\) or probable \((n = 79)\) invasive aspergillosis were studied. Isolates were available from all the patients, who were classified according to the revised criteria of the European Organisation for Research and Treatment of Cancer \(1\); patients with chronic obstructive pulmonary disease (COPD) fulfilled Bul- pa’s criteria \(7\). The isolates were obtained from pulmonary \((n = 82)\), extrapulmonary \((n = 3)\), central nervous system \(n = 1\), urinary tract \(n = 1\), and other \(n = 2\), and pulmonary and extrapulmonary \((n = 4)\), and wound and central nervous system \(n = 1\), urinary tract and central nervous system \(n = 1\) specimens. Patients with asymptomatic colonization were selected as controls; we recruited a sufficiently large number of control patients \((n = 141)\) to obtain a similar number of samples from the two groups.

A. fumigatus was isolated in 441 samples \((n = 217)\) \(49\%\) from patients with aspergillosis and 224 from colonized patients) that were cultured on bacterial and mycological media; A. fumigatus colonies \((n = 1,373)\) \(51\%\) isolates from patients with invasive aspergillosis) found on the culture plates were prospectively subcultured and stored independently. Most isolates were from the lower respiratory tract \((n = 1,227)\) \(89\%\). All the isolates were identified to the molecular level by sequencing the B-tubulin gene and were genotyped using the STRAf assay \(8, 9\). Genotypes were considered identical when they showed the same alleles for all 9 markers. A cluster was defined as different isolates with an identical genotype. Genotypic diversity was represented graphically using a minimum spanning tree. Simpson’s index of diversity \(D\) was calculated to study the genotypic diversity of A. fumigatus overall and by groups of patients with and without invasive aspergillosis \(10\).

This study was approved by the local ethics committee (Comité Ético de Investigación Clínica del Hospital Gregorio Marañón [CEIC-A1]). All patient data were anonymized.

Overall, 395 genotypes were detected in the 236 patients \(1\); 50\% of the patients yielded \(\geq 2\) genotypes \(r e a n g e, 2 \text{ to } 7\) \(2\). The overall genetic diversity and that found in the groups of the patients with and without invasive aspergillosis was 0.995, 0.988, and 0.992, respectively. Most of the genotypes \((n = 345)\) \(87\%\) were found in 1 patient each, and 153 were from patients with invasive aspergillosis, whereas 192 were from colonized patients. The remaining genotypes \((n = 50)\) \(13\%\) were clusters affecting 95 of the 236 \(40\%)\) patients \(1\); the clusters involved 2 patients \(37/50\) clusters, 3 patients \(9/50\) clusters), or 4 patients \(4/50\) clusters). Patients in the clusters were admitted to the hospital on different dates. Some clusters involved only patients with invasive...
aspergillosis (12/50 genotypes) or colonized patients (14/50 genotypes), whereas the others (24/50 genotypes) involved both groups. The percentages of patients with invasive aspergillosis or colonization in the clusters were 45% and 37%, respectively.

In order to know whether the isolates from patients with a definitive diagnosis of invasive aspergillosis were found exclusively in infected patients, 30 genotypes found in the 16 patients with proven aspergillosis were studied. However, 4 of the 30 genotypes from patients with invasive aspergillosis were also found in asymptomatic colonized patients.

FIG 1 Minimum spanning tree (MST) showing the interstrain genetic relatedness of the 395 *A. fumigatus sensu stricto* genotypes found. Only one representative isolate per genotype and per patient was chosen to construct the MST. Circles represent different genotypes. The size of each circle represents the number of isolates belonging to the same genotype. Colors indicate the sources of the isolates. Connecting lines between the circles show the similarity between profiles; solid and bold lines indicate differences in only 1 marker, a solid line indicates differences in 2 markers, long dashes indicate differences in 3 markers, and short dashes indicate differences in 4 or more markers.

FIG 2 Number of patients in whom one or more genotypes were found.
The main limitation of this study is that genes specifically related to virulence that may be better markers than STRAf were not studied. Future studies using procedures with higher resolution, such as whole-genome sequencing, will help us to discern whether there is an association between genotypes and the presence of infection or whether the clusters found by STRAf typing are a consequence of homoplasy or genetic recombination. There is growing evidence of the presence of sexual reproduction in A. fumigatus (13), and this phenomenon may limit the use of STRAf and other classic typing procedures in clinical practice.

We conclude that genotyping cannot discriminate between isolates from patients with invasive aspergillosis and those from colonized patients, as 6% of the genotypes were found in each of the groups.

ACKNOWLEDGMENTS
We thank Thomas O’Boyle for editing the article.

This study was supported by grant CP09/00055 from the Fondo de Investigación Sanitaria (FIS, Instituto de Salud Carlos III). J.G. (grant MS09/00055) and P.E. (grant CD09/00230) are supported by the FIS.

We declare no conflicts of interest.

REFERENCES


