Does monitoring *cyp51A*-mediated resistance in *Aspergillus fumigatus* by pyrosequencing lead to patient benefit?

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Purpose

The global trend of rising triazole resistance rates in *Aspergillus fumigatus* requires close monitoring.

The high volume culture (HVC) procedure developed at the NHS Mycology Reference Centre Manchester (MRCM) has improved culture positivity, thereby allowing EUCAST susceptibility testing to monitor resistance.

However, as the failure rate can be up to 70%, a molecular approach is warranted.

Commercial assays to test for the environmental mutations (e.g. TR34/L98H and TR46/Y121F/T289A) offer limited coverage of *cyp51A* polymorphisms which do not often result from long courses of azole treatment experienced by patients with progressive, chronic conditions.

Therefore, we adopted a pyrosequencing approach to monitor patients with chronic pulmonary aspergillosis (CPA) and/or allergic bronchopulmonary aspergillosis (ABPA), who demonstrated signs of clinical failure: those who demonstrated good drug levels but were persistently positive by *Aspergillus* spp. qPCR and remained symptomatic.

Methods

Over the course of three years (December 2016-2019) of testing, we assessed the impact of monitoring triazole resistance by HVC and pyrosequencing on patient outcome.

We performed a retrospective audit over a 26-month period at MRCM and the UK National Aspergillosis Centre (NAC).

We reviewed the records of 100 confirmed CPA and ABPA patients (Figure 1) to assess the impact of susceptibility and/or pyrosequencing results on patient care.

Fifty patients, who had at least one sample analysed by pyrosequencing, were considered for review alongside 50 patients who did not have the test; these patients were selected randomly but matched as closely as possible to the latter cohort.

Hospital and laboratory databases were searched for patient history, well-being scores (St. George's Respiratory Questionnaire (SGRQ) measuring Quality of Life (QoL)[1] and MRC dyspnoea scores[2]) and laboratory findings.

	Pyrosequenced		Non-pyrosequenced	
	Males	Females	Males	Females
n	32	18	32	18
CPA	20	10	23	8
CCPA	5	1	4	4
CFPA	0	1	0	0
ABPA	4	4	5	4
ABPA/CPA	3	2	0	1
ABPA/CCPA	0	0	0	1

Figure 1. Comparison of the distribution and diagnoses of 50 patients whose samples were monitored for resistance by pyrosequencing and matched with 50 patients (for age, sex and diagnosis) whose samples were monitored but not sequenced.

Results

For patients whose samples underwent pyrosequencing, there was resistance detected at **almost twice the frequency** of those patients for whom only HVC was available (10/50 versus 6/50).

Moreover, resistance was identified by pyrosequencing in **16% of HVC negative samples** in this group.

If pyrosequencing had not been used, **8 cases of resistance** would have been missed. In contrast, **no resistance was** detected in the matched group of patients.

Identification of the environmental **TR34/L98H** mutation was found only by pyrosequencing and in **6/50 patients** whose samples were pyrosequenced but **none** in the matched cohort.

	Pyrosequenced		Non-pyrosequenced		p value
QoL scores	Males (n = 28)	Females (n = 15)	Males (n = 30)	Females (n = 9)	(Males, females)
Symptom Score	76.80 ± 16.56	67.29 ± 18.48	68.80 ± 20.89	52.95 ± 22.55	0.1903, 0.2094
Impact Score	57.91 ± 24.14	54.92 ± 21.42	47.29 ± 27.07	34.81 ± 28.10	**0.0004, 0.0107**
Activity Score	78.24 ± 22.44	78.58 ± 20.27	72.35 ± 28.69	59.70 ± 32.36	0.8209, 0.0631
Total Score	67.09 ± 21.55	64.07 ± 18.15	58.11 ± 25.11	44.36 ± 28.05	0.2007, 0.1197
	Males (n = 29)	Females (n = 15)	Males (n = 25)	Females (n = 11)	(Males, females)
MRC scores	3.49 ± 1.13	3.13 ± 0.98	3.30 ± 1.36	2.36 ± 0.96	0.9192, 0.0679

Table 1. Comparison of SGRQ QoL and MRC scores between patient groups. Significance was assessed using GraphPad Prism 8.1.2 software with an unpaired t test (data were checked for normality). Significance was set at p=0.05.

Patient groups were equivalent with respect to age, sex, diagnosis (Figure 1) and weight/height (data not shown). The age distribution between groups was also equivalent (data not shown).

In this initial assessment of SGRQ and MRC data, there was significant difference in the Impact scores between patient groups (Table 1).

Surprisingly, there was higher mortality rate in the group of patients whose samples were not pyrosequenced (7/50 versus 4/50).

Conclusions

Pyrosequencing is an effective molecular method for monitoring and early detection of triazole resistance in our centre. It also has a higher sensitivity for detecting triazole resistance than culture-based susceptibility testing.

The higher QoL scores reflect the fact that pyrosequenced patients are less well, yet show better mortality outcome in this small sample set. Analysis of a larger dataset and the extended measures of disease activity (e.g. FEV1) is in progress.

Acknowledgements

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References

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