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ePoster Viewing
Highlights from molecular mycology

COMPARISON OF TWO ASPERGILLUS REAL-TIME PCR ASSAYS WITH GALACTOMANNAN TESTING OF BRONCHOALVEOLAR LAVAGE FLUID FOR THE DIAGNOSIS OF INVASIVE PULMONARY ASPERGILLOSIS

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Objectives:
Invasive pulmonary aspergillosis (IPA) in severely immunocompromised patients is associated w ith high mortality rates. Early diagnosis of IPA is difficult. PCR detection of *Aspergillus* in bronchoalveolar lavage (BAL) fluid offers a rapid and sensitive alternative to current used diagnostic techniques. We compared the results of two different PCR sets w ith galactomannan (GM) testing in BAL fluids of 18 patients.
Methods:
We used 22 BAL fluids from 18 patients that had been tested for galactomannan and stored at -80°C. After MagNa Pure DNA isolation, real-time PCR was performed w ith 2 different RT-PCR assays. PCR 1 was described in the literature, PCR 2 is used in our national reference laboratory. We w anted to detect the 5 most common *Aspergillus* species described in IPA: *A. fumigatus*, *A. niger*, *A. nidulans*, *A. terreus* and *A. flavus.* DNA of each sample w as isolated with and without bead-beating before isolation. Patients w ere categorized according to the most recent EORTC/MSG consensus definition criteria of IPA (2008).
Results:
9 GM positive (OD index >= 1,5) and 13 GM negative (culture negative) BAL fluids were analyzed (table 1). 2/9 GM positive samples w ere culture positive (*A. fumigatus*, sample 1 and 2). Because of the high sensitivity of PCR 1, negative controls frequently appeared to be positive. Therefore w e used a cut-off for PCR positivity of samples in PCR 1. A PCR w as considered to be positive if the Cp-value w as >= 1 Cp-value below the low est Cp-value of the negative controls (w e used at least 2 negative controls per run). In PCR 2 all negative controls w ere negative. Bead-beating did not influence Cp-values. In PCR 1, 8/9 GM positive samples w ere considered PCR positive. 1/13 GM negative samples was positive. In PCR 2, 7/9 GM positive samples w ere PCR positive. 3/13 GM negative samples were PCR positive.
Both PCRs w ere negative in one GM positive sample (sample 9), this patient appeared to have a fungal infection w ith a *Fusarium* spp. Both PCRs w ere positive in one GM negative sample (sample 22), this patient was diagnosed w ith bronchiectasis and previous cultures of respiratory samples grew *A. fumigatus*. To calculate test accuracy, we defined proven and probable patients as having IPA. Possible or no IPA patients and sample 9 w ere defined as not having IPA. Sensitivity for GM, PCR 1 and PCR 2 was 100%, 100% and 87.5%, respectively. Specificity w as 92.9%, 92.9% and 78.6%, respectively.
Conclusion:
For diagnosing IPA in immunocompromised patients both PCRs showed high sensitivity, according to GM testing. An *Aspergillus* specific PCR is a very useful tool to provide a rapid diagnosis of IPA. A PCR can be used in addition to GM testing in BAL fluids and it differentiates betw een IPA and other fungi.

Patient	Sample	EORTC	GM >= 1.5	Cp PCR1	Cp PCR2	Results PCR1	Results PCR2
1	1	Probable	**7.22**	24,61	28,05	pos	pos
	2	Probable	**6.71**	25,73	29,37	pos	pos
2	3	Probable	**3.13**	28,90	31,99	pos	pos
3	4	Aspergilloma	**9.47**	31,69	35,23	pos	pos
4	5	Probable	**5.70**	34,68	40,00	pos	pos
5	6	Probable	**5.70**	35,10	38,10	pos	pos
6	7	Probable	**2.70**	36,58	38,16	pos	pos
7	8	Probable	**6.10**	35,69	neg	pos	neg
8	9*	Probable	**8.80**	neg	neg	neg	neg
	10*	No	0.20	40,00	neg	neg	neg
9	11	Possible	0.40	37,28	38,64	neg	pos
10	12	No	0.20	38,51	neg	neg	neg
	13	No	0.20	38,37	neg	neg	neg
11	14	No	0.30	40,00	neg	neg	neg
12	15	No	0.20	40,00	neg	neg	neg
	16	No	0.10	40,00	neg	neg	neg
13	17	No	0.10	38,53	neg	neg	neg
14	18	Possible	0.20	38,49	neg	neg	neg
15	19	No	0.20	40,00	40,00	neg	pos
16	20	Possible	0.10	neg	neg	neg	neg
17	21	No	0.10	38,34	neg	neg	neg
18	22	No	0.60	34,13	39,53	pos	pos
	NC			38,10	neg		

Table 1.
GM = galactomannan. NC = Negative control. Cp= Cp-value.
* Time difference between sample 9 and 10 is one year.
In bold: galactomannan positive.
In red: discrepancies between the GM test and the PCR.
PCR 1: positive if Cp-value <=37,10. *Johnson et al, PLoS ONE 2012.*
PCR 2: *W.J.G. Melchers, Radboud University Nijmegen Medical Centre.*