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False-positive results in premature infants with the Platelia® *Aspergillus* sandwich enzyme-linked immunosorbent assay

Falsch-positive Ergebnisse bei Frühgeborenen mit dem Platelia®-*Aspergillus*-Sandwich-ELISA

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Schlüsselwörter: *Aspergillus*-Infektion, *Aspergillus*-ELISA, Antigen-Nachweis, Platelia®-*Aspergillus* ELISA, Frühgeborene, Mykoserologie.

Summary. The sensitivity of a sandwich enzyme-linked immunosorbent assay (ELISA) for detecting *Aspergillus* galactomannan was tested using 783 serum samples obtained from 247 patients (1–15 sera per patient) with severe underlying diseases (haematological malignancies or intensive care unit stay). We selected 146 serum samples from 50 patients for retesting. Serum samples were frozen after routine testing at −18 °C until retesting. All patient charts were checked for signs of *Aspergillus* infection, such as pneumonia or sinusitis. Adult patients were divided into four groups: proven (5), probable (6), suspected (8) or unlikely (25) *Aspergillus* infection. The results of Platelia® ELISA were 100% in proven, 33% in probable and 50% in suspected *Aspergillus* infection. Patients with unlikely infection had no positive results with Platelia® ELISA. Group 5 consists of six paediatric patients with prolonged ICU stay and a birth weight of 400–1320 g. In five out of six infants we found positive results with Platelia® ELISA. All positive results in this group of patients are considered as false positive (83.3%).

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Zusammenfassung. Die Sensitivität eines Sandwich enzyme-linked immunosorbent assay (ELISA) wurde mit 783 Sera von 247 Patienten (1 bis 15 Sera/Patient) mit schweren Grunderkrankungen überprüft (Hämatologische Grunderkrankungen oder Aufenthalt auf einer Intensivstation). Für die Nachtestung wählten wir 146 Proben von 50 Patienten aus. Nach der Routine-Testung wurden die Serum-Proben bei −18 °C bis zur Retestung eingefroren. Alle Patientenakten wurden auf Zeichen von *Aspergillus*-Infektionen wie Pneumonie oder Sinusitis hin überprüft. Die erwachsenen Patienten teilten wir in vier Gruppen ein: Gesicherte (5), wahrscheinliche (6), vermutete (8) oder unwahrscheinliche (25) *Aspergillus*-Infektion. Die Ergebnisse für den Platelia® ELISA waren 100% Sensitivität bei gesicherten, 33% bei wahrscheinlichen und 50% bei vermuteten *Aspergillus*-Infektionen. Bei Patienten in Gruppe 4 (unwahrscheinliche Infektion) kamen keine positiven Ergebnisse vor. Gruppe 5 bestand aus 6 pädiatrischen Patienten mit langdauerdem Aufenthalt auf einer Intensivstation und einem Geburtsgewicht von 400 bis 1320 g. Bei 5 von 6 Kindern fanden wir positive Ergebnisse mit dem Platelia® ELISA. Alle positiven Ergebnisse mußten als falsch-positive eingeordnet werden.

Introduction

Invasive aspergillosis is a frequent cause of death in neutropenic patients undergoing chemotherapy

or bone marrow transplantation. Inhalation of conidia results in pulmonary infection. Dissemination to other organs occurs. In the early stage of infection specific signs of invasive aspergillosis are rare. Fever despite broad spectrum antibiotic treatment may be the first sign of *Aspergillus* infection, but ≈30% of all patients with pulmonary aspergillosis present no pathological findings in radiographs of the thorax [1]. Serodiagnosis of circulating galactomannan (CGM) is a helpful tool in diagnosis of invasive aspergillosis, but sensitivity of latex agglutination was poor [2–4]. The detection threshold of CGM was 15 ng ml^{-1} [5]. In a new sandwich enzyme-linked immunosorbent assay for detection of CGM the threshold is reduced to 1 ng ml^{-1} [5]. Sensitivity was enhanced to 90% [3, 4]. Enhancement of sensitivity is a risk for lower specificity. False-positive reactions may occur [5, 6].

Materials and methods

Patients

From February to October 1997, all serum samples from routine screening programmes were frozen at −18 °C until retesting. We selected 146 sera from 50 patients (1–10 samples per patient) for retesting. All samples selected were from patients with positive histology, radiology and cultures, or at least one positive serum in routine serology, and all sera from premature infants. Patients charts of the selected patients were obtained from the archives. Autopsy protocols of all deceased patients during the period (February–October 1997) were checked for the diagnosis of *Aspergillus* infection. Patients were divided into four groups: (1) proven infection, patients with clinical signs of aspergillosis and positive histology (five patients); (2) probable infection, patients with clinical signs of aspergillosis and positive cultures (six patients); (3) suspected infection, patients with clinical signs of aspergillosis and positive radiology without detection of pathogens in pulmonary specimen (eight patients); (4) unlikely infection, patients without clinical signs of aspergillosis (25 patients); and (5) paediatric patients (six patients). Patient characteristics of groups 1, 2, 3 and 5 are shown in Table 1.

ELISA

Platelia® *Aspergillus* ELISA was obtained from a single batch (7G015U/expiry date March 1998) from Sanofi Pasteur, Freiburg, Germany. Retesting of sera was performed exactly according to the manufacturer's instructions. Positive and negative control sera were tested in duplicate. Cut-off sera were tested four times. Reading of plates was performed in a Dynatech MRX Microplate Reader, Dynatech, Denkendorf, Germany at lambda 450 nm and 630 nm.

Results

We tested 783 serum samples in routine screening programmes: 44 samples with positive (5.6%) CGM (index > 1.5) and 18 with doubtful (2.3%) results (index, 1.0–1.5). Retest results, 29 patients turned from positive (or doubtful) to negative, including one patient with probable aspergillosis. Serum samples from 18 patients with CGM index > 1.0 in routine testing remained > 1.0. One patient turned from negative (Index 0.91) to doubtful (Index 1.19). Table 2 shows the results of patients with proven, probable and suspected *Aspergillus* infection, and the results of samples from paediatric patients. All patients with proven aspergillosis showed positive CGM results during their infection. Group 5 consists of six premature infants. One child had a negative result and five children had positive results (one to three positive samples).

Discussion

The enhanced sensitivity of the Platelia® ELISA may lead to false-positive reactions in routine testing. In our serology laboratory, we pay attention to the most common reasons for false-positive reactions: we do not proceed cultures in the serology laboratory and indoor plants are not present. We keep test tubes and microtitre plates in plastic containers to avoid prolonged air contact and contamination with dust. Nevertheless, false-positive results occur. Positive results should be confirmed by retesting the first positive sample and testing a second specimen, as proposed in the instruction leaflet of the manufacturer.

The test results of specimens from adults in our evaluation showed 100% sensitivity in proven *Aspergillus* infections. Sensitivity of CGM results was superior to culture and radiology. Both of these methods failed to detect aspergillosis in patient 5 of this group.

Patients with probable aspergillosis showed different results. Only three patients with probable aspergillosis had detectable CGM (patients 6, 7 and 24). Patients 2 and 7 died. In their autopsy samples *Aspergillus* spp. could not be detected macro- or microscopically. It is possible that they had, despite some positive cultures, a non-invasive infection. The negative or doubtful CGM results

Table 1. Summary of patient characteristics

Patient number	Sex	Age (years)	Ward	Underlying disease and/or condition	Group character
1	Female	76	ICU	Lung fibrosis, steroid therapy	Patients with proven aspergillosis
3	Male	66	ICU	NHL, PCT	
4	Male	30	Medical	HD, BMT	
5	Female	27	ICU	MDS, BMT, pancytopenia, GVHD, steroid therapy	
17	Female	74	ICU	Alcoholism, pancreatitis, steroid therapy	
2	Male	68	ICU	Listeria meningitis, alcoholism	Patients with probable aspergillosis
6	Male	53	Medical	AML, PCT, neutropenia	
7	Female	77	ICU	Intra-abdominal abscess, surgery, sepsis	
8	Male	75	Medical	Tuberculosis, vasculitis, steroid therapy	
24	Female	38	ICU	AML, PCT, neutropenia	
25	Male	56	Medical	AML, PCT, neutropenia	
9	Female	38	ICU	NHL, BMT, PCT	Patients with suspected aspergillosis
10	Female	37	ICU	NHL, BMT, PCT	
11	Female	63	Medical	ALL, PCT, neutropenia	
12	Male	68	Medical	MDS, COPD, PCT, neutropenia	
13	Male	57	Medical	AML, PCT, neutropenia	
14	Female	21	Medical	HD, BMT, PCT, neutropenia	
15	Male	53	Medical	AML, PCT, neutropenia	
16	Female	39	Medical	AML, PCT, neutropenia	
Patient number	Sex	Age (days)	Ward	Underlying disease and/or condition	Premature infants
18	Female	58	ICU	26th week of gestation, birth weight 800 g, RDS III°	
19	Male	19	ICU	24th week of gestation, birth weight 800 g	
20	Male	19	ICU	28th week of gestation, birth weight 1095 g, RDS III°	
21	Male	19	ICU	28th week of gestation, birth weight 1300 g	
22	Female	49	ICU	24th week of gestation, birth weight 525 g, RDS I°	
23	Male	136	ICU	24th week of gestation, birth weight 400 g, RDS IV°	

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BMT, bone marrow transplantation; COPD, chronic obstructive pulmonary disease; GVHD, graft-versus-host disease; HD, Hodgkin's disease; ICU, intensive care unit; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; PCT, polychemotherapy; RDS, respiratory distress syndrome.

go well together with colonization. Patient 25 developed infection after neutropenia and survived with cAmB therapy. His sera showed high antibody titres (IHA (indirect immunohemagglutination): $>1:640$). CGM was not detectable in both sera that we received. The last patient in this group without positive CGM results was patient 8. Only one of seven cultures yielded *Aspergillus niger*. Although he improved under itraconazole (the only anti-infective agent he received), it is possible that we cultured a contaminant or a temporary colonization. False-positive reactions did not occur in the groups with adult patients. The results of our evaluation for adult patients are similar to other evaluations [7].

Rohrlich *et al.* [6] evaluated the Platelia® ELISA with 37 paediatric patients. In their study, only 12 false-positive results (5.7%) occurred. The age of their patients ranged from 5 months to 18 years. All patients were neutropenic under chemotherapy or were recipients of bone marrow transplantation (BMT). Our test and retest results of sera obtained from premature infants are not explainable. Five of the infants had no sign of *Aspergillus* infection, but four of them had positive CGM results. One child (patient 22) had a positive blood culture. The blood culture yielded *Candida albicans*. She received antifungal therapy and survived her infection. A case of fungaemia with *Candida albicans* and a false-positive result using Platelia® ELISA had been reported before [8]. Another child (patient 20) with clinically suspected fungal infection received antifungal therapy (cAmB). A blood culture and two tracheal aspirates remained sterile. PCR results from tracheal aspirate for *Adenovirus*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Parainfluenzavirus 1+3*, *RSV* and *Enterovirus A+B* were negative. His CGM after the course of therapy remained positive, and he died during a second episode of sepsis.

Autopsy results: Lung, kidney, liver, spleen and brain were free of *Aspergillus* spp. Cause of death was bronchopulmonary dysplasia. Two of the other three children survived without antifungal therapy. The last child received cAmB for fever under

Table 2. Diagnosis of invasive pulmonary aspergillosis

Patient number	Outcome	Autopsy	Microbiology	Culture of BAL	Tracheal aspirate	Sputum	Serology CGM Index	Radiology
		Aspergillus in:	Cultured species:					
Group 1: proven aspergillosis								
1	Deceased	Lung	*Aspergillus fumigatus*	ND	2 × Positive	1 × Positive	4.24 4.06	Bilateral lung infiltrates
3	Deceased	Lung	*Aspergillus fumigatus*	1 × Positive	1 × Positive	ND	4.74	Bilateral lung infiltrates
4	Deceased	Lung, brain, kidney	*Aspergillus fumigatus**	ND	ND	ND	2.81	CT: cavitation, multifocal lung infiltrates, pleural effusion
5	Deceased	Lung**, brain, kidney	*Candida krusei****	1 × Negative	2 × Negative	ND	5.44 4.66 5.08 5.59 6.21	No abnormalities
17	Deceased	Lung, trachea	*Aspergillus fumigatus*	ND	2 × Positive	ND	6.30	Bilateral lung infiltrates
Group 2: probable aspergillosis								
2	Deceased	Not detected	*Aspergillus fumigatus*	ND	2 × Positive, 1 × Negative	ND	0.31 0.45 0.44	Bilateral lung infiltrates
6	Survived		*Aspergillus flavus*	1 × Positive	ND	ND	1.88 0.67	CT: cavitation, multifocal lung infiltrates
7	Deceased	Not detected	*Aspergillus fumigatus*	ND	3 × Positive	ND	0.18 0.35 0.17 0.47 0.63 1.19	Unifocal lung infiltrates
8	Survived		*Aspergillus niger*	1 × Negative	ND	1 × Positive, 5 × Negative	0.16 0.22	CT: lung fibrosis, bullous changes, emphysema, bronchitis
24	Deceased	Not done	*Aspergillus fumigatus*	ND	1 × Positive, 2 × Negative	3 × Negative	4.51 4.35 4.09 4.33 5.89	Bilateral lung infiltrates
25	Survived		*Asp. flavus + fumigatus*	1 × Positive	ND	ND	0.20 0.82 0.13 0.11	CT: cavitation, multifocal lung infiltrates
Group 3: suspected aspergillosis								
9	Deceased	Not done		1 × Negative	2 × Negative	ND	2.24	Bilateral lung infiltrates
10	Deceased	Not done		1 × Negative	5 × Negative	ND	1.17 1.28 0.63	Pleural effusion, interstitial infiltrates
11	Deceased	Not done		ND	ND	ND	3.35	CT: unifocal lung infiltrates, air crescent
12	Survived			ND	ND	ND	1.36	Unilateral lung infiltrates
13	Deceased	Not done		ND	ND	ND	1.68	Bilateral lung infiltrates
14	Survived			1 × Negative	ND	4 × Negative	0.21 0.17	CT: multifocal lung infiltrates
15	Deceased	Not done		ND	2 × Negative	2 × Negative	0.19 0.15 0.60 4.35	Bilateral lung infiltrates
16	Survived			ND	ND	2 × Negative	0.14 0.27 0.19 0.51 0.95 0.27 0.30	CT: bilateral lung infiltrates
Group 4: paediatric patients								
18	Survived			ND	2 × Negative	ND	5.83	No abnormalities
19	Survived			ND	1 × Negative	ND	3.14 2.29 6.21	ND
20	Deceased		Not detected	ND	2 × Negative	ND	3.13 1.15	Unilateral lung infiltrates
21	Survived			ND	5 × Negative	ND	1.66	ND
22	Survived		*Candida albicans*****	ND	1 × Negative	ND	1.80 0.68	ND
23	Survived			ND	2 × Negative	ND	0.19	ND

BAL, bronchoalveolar lavage; CGM, circulating galactomannan; ND not done. *Autopsy material: lung tissue; **_Aspergillus_ and _Candida_; ***Blood culture, CVC-tip, bronchial aspirate (2 ×); ****Blood culture.

broad spectrum antibiotic therapy. The chest radiograph showed no sign of aspergillosis, and two tracheal aspirates remained sterile for fungi. Aspergillosis was considered as unlikely. In premature infants we found five out of six patients (83.3%) having false-positive CGM results. The reason remains unclear, because the therapy and nutrition of the child with negative CGM did not differ basically from those of the CGM-positive children. The differences consisted of distinct antibiotic treatment regimens.

Conclusion

The Platelia® ELISA in our evaluation showed 100% sensitivity in proven invasive *Aspergillus* infection in adults. False-positive test results occurred in testing samples from premature infants. It is necessary to evaluate the Platelia® ELISA for this group of patients. Despite the possibility of early diagnosis in invasive aspergillosis using Platelia® ELISA, the test did not change the outcome of our patients. All patients remaining in neutropenia died, despite early onset of antifungal therapy. The most important factors in invasive aspergillosis remain prophylaxis and improvement of therapy.

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