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In-vitro detection of mannan and galactomannan in components of total parenteral nutrition (TPN)

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Detection of *Candida* mannan and *Aspergillus* galactomannan in serum with the Platelia™ enzyme immunoassay is applied for diagnosing invasive fungal infections. High risk patients for invasive fungal infections are often receiving parenteral nutrition. It is important to know whether false-positive Platelia™ test results occur during total parenteral nutrition. Studies to false-positivity in intravenous feeding solutions lack so that we start an *in-vitro* investigation. We used two different enzyme immunoassays to test the feeding solutions. We tested infusions (n=19) which are applied for the production of individual parenteral nutrition in the University Hospital Carl Gustav Carus Dresden. We used the Platelia™ *Aspergillus* EIA to analyse the *Aspergillus* antigen galactomannan in the solutions. In addition, the Platelia™ *Candida* Ag plus was used to determine the concentration of the *Candida* antigen mannan. In summary, four solutions (21 %) showed measurable concentrations of the *Candida* mannan. They were considered positive with a concentration > 0.125 ng/ ml mannan (Tracitrans® infant, calcium gluconate solution) and borderline with a concentration between 0.0625 and 0.125 ng/ ml mannan (Tracitrans® plus, SMOFlipid®). None of the analysed infusions contained the *Aspergillus* galactomannan. In conclusion, further investigations on the topic are necessary to determine their *in-vivo* impact. A positive Platelia™ test result can simulate the presence of invasive fungal infections. As a consequence the patient may be treated with expensive, systemic antimycotics with a high risk of adverse events. Therefore a definite diagnosis is important.

1. Introduction

Opportunistic pathogens like *Candida* and *Aspergillus sp*. may cause invasive fungal infections (IFI) especially in immunocompromised patients. Invasive candidiasis shows a mortality rate of 40-50 % (Eggimann et al. 2011) and invasive aspergillosis a rate of 40-80 % (Kedzierska et al. 2007). Due to the absence of typical symptoms the diagnosis is difficult. The use of an enzyme-linked immunosorbent assay (ELISA) named Platelia™ is an opportunity to diagnose invasive fungal infections (IFI). The fungal cell wall contains polysaccharide antigens. The increase of these antigens in the blood can be a marker for IFI. Circulating polysaccharides like mannan and galactomannan can be detected in an antigen-antibody- reaction of this ELISA before clinical symptoms occur (Swanink et al. 1997). The antigen mannan is a laboratory marker for invasive candidiasis (Platelia™ *Candida* EIA plus). The diagnosis of invasive aspergillosis is based on the detection of galactomannan (Platelia™ *Aspergillus* EIA). In addition, a positive Platelia™ *Aspergillus* EIA result represents a criterion according to the European Organization for Research and Treatment of Cancer-Mycoses Study Group (EORTC-MSG) for a probable invasive mycosis (Wild et al. 2001; Huppmann 2010). Various studies show false-positive Platelia™ test results caused by concurrent administration of intravenous drugs, therefore the value of the test has been questioned. Previously, Viscoli et al.(2004) reported false-positive *in-vivo* Platelia™ test results for *Aspergillus* in piperacillin-tazobactam. The reason is the antigen galactomannan. Detection of galactomannan in antibiotics originates from cross-reactivity with *Penicillium* antigens and the Platelia™ test. *Penicillium* species are used for production of semisynthetic drugs like piperacillin-tazobactam. Since the detection of fungal antigens in gluconate solution (Plasma-Lyte®) (Hage et al. 2007), infusion solutions became the focus of attention.

This study intends to test total parenteral nutrition (TPN) which is usually infused continuously in high volumes. The circulating antigens simulate the existence of fungal cells but do not cause pathogenicity. As a consequence an antimycotic treatment may be started. High risk patients for IFI are often multimorbid and TPN-nourished so that a diagnosis has to be definite. Systemic antimycotics like liposomale amphotericine B or caspofungine show serious side effects so that the application has to be justified. This study gives an overview about components of TPN and summarizes infusions which are positively tested *in vitro* with the Platelia™ at the University Hospital Dresden (Walter 2014).

2. Investigations and results

The infusions were tested as prescribed below. Each infusion solution was classified as positive, borderline or negative after appraisal of all three samples. The results of the Platelia™ *Candida* Ag plus are presented in table 2. A definite negative test result means that every sample was tested negative in every test round. In summary, eight infusion solutions showed such a definite negative test result by using the Platelia™ *Candida* Ag plus to detect the antigen mannan: Infesol® 10 %, Aminoplasmal® 10 % Hepa, Aminopäd®, potassium chlorid solution 1M (vial), sodium glycerophosphat, sodium bicarbonate solution 8,4 %, glucose 40 % infusion solution and Uromitexan® multidose. Furthermore we appraised the entire result of a sample negative by having at least two negative Platelia™ *Candida* test results. A sample is considered borderline when there are at least two borderline test results. In addition a sample was classified as positive when the test results of at least two test rounds were positive. These rules were applied on the following solutions: The first sample of glucose 5 % was tested borderline (2 borderline test results, 1 negative test result), the

Test vials, batch	Test 1		Test 2		Test 3		Appraisal	
	c_{Man} [1]	Appraisal	c_{Man} [1]	Appraisal	c_{Man} [1]	Appraisal	Per sample	Total
Aminopäd 10 % *sample 1: 13F0381*	0.00	N	0.00	N	0.00	N	**N**	**N**
sample 2: 13F0381	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 13F0381	0.00	N	0.00	N	0.00	N	**N**	
Aminoplasmal 10 % Hepa *sample 1: 124018062*	0.00	N	0.00	N	0.00	N	**N**	**N**
sample 2: 124018062	0.00	N	0.00	N	0.00	N	**N**	
Calcium gluconate 10 % injection solution *sample 1: 13252011*	> 0.50	P	> 0.50	P	> 0.50	P	**P**	**P**
sample 2: 13252011	> 0.50	P	> 0.50	P	> 0.50	P	**P**	
sample 3: 13252011	> 0.50	P	> 0.50	P	> 0.50	P	**P**	
Glucose 5 % *sample 1: 14306P1-1*	0.10	B	0.08	B	0.06	N	**B**	**N**
sample 2: 14306P1-1	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 14306P1-1	0.00	N	0.00	N	0.00	N	**N**	
Glucose infusion solution 40 % *sample 1: 00312*	0.00	N	0.00	N	0.00	N	**N**	**N**
sample 2: 00313	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 00313	0.00	N	0.00	N	0.00	N	**N**	
Infesol 10 % *sample 1: 24017021*	0.00	N	0.00	N	0.00	N	**N**	**N**
sample 2: 24017021	0.00	N	0.00	N	0.00	N	**N**	
Magnesium Verla *sample 1: 130008*	0.20	P	0.14	P	0.10	B	**P**	**N**
sample 2: 130008	0.06	B	0.00	N	0.00	N	**N**	
sample 3: 130008	0.00	N	0.08	B	0.00	N	**N**	
Potassium chloride solution 1M 7.45 % (bottle) *sample 1: 00413*	0.10	B	0.07	B	0.00	N	**B**	**N**
sample 2: 00413	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 00413	0.00	N	0.00	N	0.00	N	**N**	
Potassium chloride solution 1M 7.45 % (vial) *sample 1: 20FHE003*	0.00	N	0.00	N	0.00	N	**N**	**N**
sample 2: 20FHE003	0.00	N	0.00	N	0.00	N	**N**	
SMOFlipid 20 % *sample 1: 16GK0168*	0.20	P	0.00	N	0.14	P	**P**	**B**
sample 2: 16GK0168	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 16GK0168	0.00	N	0.07	B	0.08	B	**B**	
Sodium bicarbonate solution 8.4 % *sample 1: 134468062*	0.00	N	0.00	N	0.00	N	**N**	**N**
sample 2: 134468062	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 134468062	0.00	N	0.00	N	0.00	N	**N**	
Sodium chloride solution 154 *sample 1: 20GCE008*	0.00	N	0.07	B	0.08	B	**B**	**N**
sample 2: 20GCE008	0.00	N	0.00	N	0.00	N	**N**	
Sodium chloride solution 1M 5.85 % *sample 1: 19GG11GB*	0.07	B	0.07	B	0.00	N	**B**	**N**
sample 2: 19GG11GB	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 19GG11GB	0.00	N	0.00	N	0.00	N	**N**	
Sodium glycerophosphat concentrate *sample 1: 20GEE019*	0.00	N	0.00	N	0.00	N	**N**	**N**
sample 2: 20GEE019	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 20GEE019	0.00	N	0.00	N	0.00	N	**N**	

Test vials, batch	Test 1		Test 2		Test 3		Appraisal	
	$c_{Man}{}^{1}$	Appraisal	$c_{Man}{}^{1}$	Appraisal	$c_{Man}{}^{1}$	Appraisal	Per sample	Total
Tracitrans Infant *sample 1: 12GFL28/1*	> 0.50	P	> 0.50	P	> 0.50	P	**P**	**P**
sample 2: 12GFL28/1	> 0.50	P	> 0.50	P	> 0.50	P	**P**	
sample 3: 12GFL28/1	> 0.50	P	> 0.50	P	> 0.50	P	**P**	
Tracitrans plus *sample 1: 90GE192*	0.20	P	0.14	P	0.16	P	**P**	**B**
sample 2: 90GE192	0.09	B	0.12	B	0.13	P	**B**	
sample 3: 90GE192	0.1	B	0.12	B	0.13	P	**B**	
Uromitexan multidose *sample 1: 3E004*	0.00	N	0.00	N	0.00	N	**N**	**N**
sample 2: 3E004	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 3E004	0.00	N	0.00	N	0.00	N	**N**	
Water for injection (B Braun) *sample 1: 13131015*	0.13	P	0.13	P	0.10	B	**P**	**N**
sample 2: 13131015	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 13131015	0.00	N	0.00	N	0.00	N	**N**	
Water for injection (Diprom) *sample 1: 13123-3*	0.14	P	0.16	P	0.10	B	**P**	**N**
sample 2: 13123-3	0.00	N	0.00	N	0.00	N	**N**	
sample 3: 13123-3	0.00	N	0.00	N	0.00	N	**N**	

Table 1: Results of *Candida* mannan EIA. The table presents the *Candida* antigen concentrations $c_{Man}{}^{1}$ of every tested sample and every test round in ng/ ml. Every result is considered positive (P), borderline (B) or negative (N) in the following column ("appraisal"). The two final columns summarize the appraisals per sample and per infusion solution in total. A positive result (P) is defined by a mannan concentration over 0.125 ng/ ml, a borderline result (B) by a mannan concentration between 0.0625 ng/ ml and 0.125 ng/ ml and a negative result by a mannan concentration below 0.0625 ng/ ml. Mannan concentrations over 0.5 ng/ ml are outside the measuring range.

two other samples showed a negative test result. One sample of Magnesium Verla was considered positive (2 positive test results, 1 borderline test result) and the other two samples were considered negative (2 negative test results, 1 borderline test result). Sample 1 of potassium chloride solution (vial) was also tested borderline (2 borderline test results, 1 negative test result) but the two other samples were definite tested negative. There is also noted a borderline tested sample of sodium chloride solution 1M 5.85 % (2 borderline results, 1 negative result) and two negative tested samples. Similar results were noticed by testing both solutions of water for injection (B Braun, Diprom). One sample was tested positive (2 positive results, 1 borderline result) and two samples were tested negative. In conclusion, the named solutions (Water for injection- Diprom/ B Braun, glucose solution 5 %, potassium chlorid solution- bottle, sodium chlorid solution 1M, magnesium Verla) have been classified as negative because the majority of the results were negative. There are two feeding solutions with a totally positive test result. Calcium gluconate solution (3 positive samples) and Tracitrans® infant (3 positive samples) showed a definite positive test result with a mannan concentration over 0.5 ng/ ml. This mannan concentration was outside the measuring range and could not be closer specified. For SMOFlipid there is one positive, one negative and one borderline sample so that it was total considered borderline. Tracitrans® plus indicated only one positive sample but two borderline samples. Therefore we considered Tracitrans® plus borderline.

In contrast to the *Candida* ELISA, the Platelia™ *Aspergillus* EIA indicated only one positive test result of nineteen tested solutions. A galactomannan index over 0.5 was classified as positive. A sample of water for injection (B Braun) showed a galactomannan index of 1.4 in one of three ELISA stages. The other two tested samples were tested negative in all three test rounds. In summary, the solution of water for injection (B Braun) is classified as negative because only a single positive test result occurred. In conclusion, there is no detection of the *Aspergillus* antigen galactomannan in the tested feeding solutions. Thus all intravenous solutions are negative for galactomannan.

3. Discussion

In conclusion there are many positive results by testing the feeding solutions with the Platelia™ *Candida* Ag plus. Calcium gluconate solution, SMOFlipid, Tracitrans® infant and Tracitrans® plus showed measurable concentrations of the *Candida* antigen mannan. According to the manufacturer's instruction we considered the test results of calcium gluconate solution and Tracitrans® infant positive (>0.125 ng/ ml mannan). The results of Tracitrans® plus and SMOFlipid were classified as borderline (0.0625 ng/ ml<c_{Man}<0.125 ng/ ml mannan). A deficiency of this study is the investigation of only one batch. False-positive Platelia™ test results can depend on the batch. Therefore the following statement can only be applied on the tested batch: These four feeding solutions can lead to false-positive Platelia™ *Candida* EIA test results *in-vitro*. Apart from the definite positive and negative tested infusion solutions several single positive test results occurred. The samples were taken under sterile conditions why a contamination in this part of the study should be excluded.

Orlopp et al.2008 reported that at least two following galactomannan tests have to be positive when the test is used for diagnosing IFI. This recommendation was applied on utilisation of the Platelia™ *Candida* Ag plus and the Platelia™ *Aspergillus* EIA. Three samples of each solution were taken, one sample more than recommended. Furthermore the manufacturer's instruction was used for the classification of the mannan concentrations. Some mannan concentrations were near the limit between a positive/ borderline result or a borderline/negative test result. This is to bear in mind for the reproduction of the results. The samples of Tracitrans® plus could be regarded as positive. The manufacturing of xylitol represents a possible cause for false-positive Platelia™ *Candida* test results. Xylitol is an excipient in Tracitrans® plus. Aside from catalytic hydrogenation, a biotechnological method using yeasts like *Saccharomyces cerevisiae* or *Candida spp.* is applicable (Bracher 2012; Fouad et al. 2007). Antigens from these yeasts can survive the manufacturing process and be found in the final product. As a consequence false-positive *Candida* results

can occur. But this fact does not explain the high concentration of mannan in Tracitrans® infant. This trace element solution lacks xylitol. Sodium gluconate solutions like Plasmalyte® are partly obtained from *A.niger* (Guige et al 2013; Surmont and Stockman 2007). Therefore a positive *Aspergillus* ELISA result by testing calcium gluconate solution (B Braun) was expected but it was negative. The calcium gluconate solution showed definite positive Platelia™ *Candida* test result. To our knowledge this is the first investigation into this matter therefore further studies are needed.

There were no positive tests of the nineteen intravenous solutions with the Platelia™ *Aspergillus* EIA. The presence of positive Platelia™ *Candida* test results did not refer with the occurrence of positive Platelia™ *Aspergillus* test results. Since the discovery of the applied antibody EB-A2 in 1992, cross-reactivity of Platelia™ *Aspergillus* EIA with various antigens is well-known (Walsh et al. 2004).The similarity of galactomannan to the antigen of *Penicillium* species is mentioned in the manufacturer's instructions of the EIA and explains false-positivity in piperacillin-tazobactam (BioRad 2009).

Polysaccharides like galactomannan are also found in fungi and plants (soya beans) (Stynen et al. 1992). In contrast to other studies concerning false-positivity with the Platelia™ *Aspergillus* EIA there are no measurable galactomannan indices in the tested TPN. This study analysed only *in-vitro* infusion solutions, the transfer from these data to a predication after infusion of TPN is not proven. So it is necessary to determine their *in-vivo* impact in further investigations. The pharmacokinetics in the human body like dilution of the antigen in blood circulation were not considered. In conclusion further investigations on this topic will help to estimate the usefullness of Platelia™ *Candida* Ag plus and *Aspergillus* EIA in the diagnosis of IFI. Especially on immunocompromised patients, who are often drip-feeded, physicians need a rapid and safe diagnosis.

4. Experimental

The parenteral nutrition were prepared according to the EU-GMP guideline (BMG 2014). Nineteen intravenous solutions were chosed which are applied for compounding individual parenteral nutrition in the pharmacy department of our hospital (Table 2).

Table 2: Solutions tested

Intravenous solution, batch	No. of samples	Manufacturer	Dosage form		
Aminopäd® 10 % *13F0381*	3	Baxter	250	ml	infusion bottle
Aminoplasmal® 10 % Hepa *124018062*	2	B Braun	500	ml	infusion bottle
Calcium gluconate 10 % injection solution *13252011*	3	B Braun	10	ml	vial
Glucose 5 % *14306P1-1*	3	AlleMan Pharma	1000	ml	infusion bottle
Glucose infusion solution 40 % *00312* *00313*	1 2	Serumwerk Bernburg	500	ml	infusion bottle
Infesol® 10 % *24017021*	2	Berlin- Chemie	500	ml	infusion bottle
Magnesium Verla® *130008*	3	Verla	10	ml	vial
Potassium chloride solution 1M 7.45 % *00413*	3	Serumwerk Bernburg	500	ml	infusion bottle
Potassium chloride solution 1M 7.45 % *20FHE003*	2	Fresenius Kabi	20	ml	vial
SMOFlipid® 20 % *16GK0168*	3	Fresenius Kabi	500	ml	infusion bottle
Sodium bicarbonate solution 8.4 % *134468062*	3	B Braun	250	ml	infusion bottle
Sodium chloride solution 154 *20GCE008*	2	Fresenius Kabi	1000	ml	infusion bottle
Sodium chloride solution 1M 5.85 % 19GG11GB	3	Fresenius Kabi	20	ml	vial
Sodium glycerophosphat concentrate *20GEE019*	3	Fresenius Kabi	20	ml	vial
Tracitrans® Infant *12GFL28/1*	3	Paesel+ Lorei GmbH	10	ml	vial
Tracitrans® plus *90GE192*	3	Fresenius Kabi	10	ml	vial
Uromitexan® multidose *3E004*	3	Baxter	50	ml	infusion bottle
Water for injection *13131015*	3	B Braun	10	ml	vial
Water for injection *13123-3*	3	Diprom	100	ml	infusion bottle

Three samples of different bottles/ vials were each taken except Aminoplasmal® 10 % Hepa, Infesol 10 %, sodium chloride solution 154 and potassium chloride solution (vial). There only two samples were tested. Apart from one exception (glucose infusion solution 40 %: 2 samples of the same batch plus 1 sample of a different batch) were tested. The samples were taken under sterile conditions in the pharmacy department of the UKD to minimise contaminations. Afterwards they were tested in the institute for microbiology. Every sample was tested three times compliant with the manufacturer's instructions of the Platelia™ test (BioRad 2011, 2009). It was an inter-serial investigation so that every sample was tested three times with different test kits. The Platelia™ *Aspergillus* EIA was used to detect the galactomannan index. The immunological reaction between antigen (galactomannan) and antibody (contained in the Platelia™ EIA) is analysed photometrical. The Platelia™ *Aspergillus* EIA results were determined as the index between the optical density of the sample and the optical density of the threshold positive control. The index is represented as a ratio without unit. The cut-off point was set at 0.5 by the manufacturer (Biorad 2009). All samples with an index below 0.5 were considered negative. The Platelia™ *Candida* Ag plus was used to measure the concentration of the antigen mannan in the nineteen infusions. The antigen-antibody reaction is also based on a photometric analysis. Both testing methods are applied in the UKD for serial testing of blood samples. A positive *Candida* mannan test was characterized by a concentration > 0.125 ng/ ml mannan. A result between 0.0625 and 0.125 ng/ ml mannan was considered borderline. All concentrations below 0.0625 ng/ ml were negative (BioRad 2011). Each result was classified as positive, borderline or negative.

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