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Research Article

Soluble Intercellular Adhesion Molecule- (sICAM-) 1, Thrombospondin-1, and Vinculin for the Identification of Septic Shock Patients Suffering from an Invasive Fungal Infection

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Background. Nowadays, invasive fungal infections (IFI) are of increasing importance and associated with an increased mortality. However, reliable diagnostic tools for the identification of patients suffering from an IFI are rare and associated with relevant weaknesses. *Methods*. Within this secondary analysis of an observational clinical study, an innovative biomarker panel (consisting of 62 biomarkers in total) was screened for the identification of septic shock patients suffering from an IFI. Fungal growth in blood cultures, intraoperative swabs, and *Aspergillus* spp. in deep respiratory tract specimens with accompanying pulmonary infiltrates were classified as infection, whereas *Candida* spp. in the respiratory tract or in fluids from drainages were classified as colonization. Plasma samples of 50 septic shock patients at six predefined timepoints within a period of 28 days following the onset of septic shock were available. *Results*. In total, 11 out of the 50 patients (22%) were shown to suffer from an IFI, whereas 22 patients (44%) presented with a fungal colonization. Within the presented biomarker panel, plasma levels of soluble intercellular adhesion molecule- (sICAM-) 1, thrombospondin-1, and vinculin were shown to be the most promising. sICAM-1 was shown to be increased in patients with an IFI, whereas thrombospondin-1 and vinculin revealed decreased plasma levels as compared to colonized patients as well as patients without any fungal findings at any time. *Conclusion*. Plasmatic measurements of sICAM-1, thrombospondin-1, and vinculin may help to facilitate the diagnosis of an IFI in human septic shock and to identify patients with an increased risk for an IFI. This trial is registered with DRKS00005463.

1. Introduction

Sepsis represents a dysregulated host response to infection and is most frequently caused by bacteria, whereas fungal or viral infections are less common [1, 2]. Although only 3% of unselected septic patients suffer from fungemia [1], fungi are one of the most often isolated species in intra-abdominal samples of patients with peritonitis. Moreover, numerous patients reveal a fungal colonization at different body sites during their hospital stay [1, 3].

Although being a rare disease entity, the incidence of invasive fungal infections (IFI) is continuously increasing and ranges between 30 and 40% in critically ill patients [4, 5]. This is due to multiple causes, such as an increasing number of immunocompromised patients or more invasive surgical procedures in the elderly as well as in high-risk

patients [1, 6–8]. Within this context, the following species seem to be most relevant: *Candida* spp. (*C. albicans*, *C. glabrata*, and *C. krusei*) in up to 80% of IFI cases [9], *Aspergillus* spp. (*A. fumigatus*), *Mucor* spp., *Rhizopus* spp. (*R. microsporus*), and *Cryptococcus* spp. [10–13]. In a worldwide ICU prevalence study, *Candida* spp. represented the third most frequently isolated microorganisms accounting for 17% of all bloodstream infections [14]. Although *Candida* spp. are responsible for no more than 5% of all cases with sepsis or septic shock, the occurrence of candidemia is associated with a septic disease course in 10–40% of cases [15]. Moreover, the incidence of invasive aspergillosis (IA) in the ICU was up to 53% in 563 patients revealing a positive culture with *Aspergillus* spp. [16]. Most interestingly, sepsis-associated mortality in patients suffering from an IFI is increased in comparison to non-IFI patients and amounts up to 42% for *Candida* spp. and is even higher in patients presenting with an IA [17–19].

Common diagnostic procedures for the identification of patients suffering from an IFI (such as culture-based diagnostics or plasma levels of β-D-glucan (BDG)) are associated with relevant weaknesses, so that a considerable number of IFI cases might be missed [20–22]. Moreover, this diagnostic insufficiency is associated with a delayed initiation of antifungal therapy, associated with the abovementioned increased mortality rates [23]. New diagnostic approaches such as polymerase chain reaction- (PCR-) or even next-generation sequencing- (NGS-) based methods have yet to demonstrate, whether they are of value for the detection of an IFI in daily clinical use [21, 24]. Therefore, there is an urgent need for innovative fungal biomarkers, reliably identifying septic patients suffering from an IFI and guiding antifungal therapy in these patients.

The aims of these secondary analyses of a previously published study of our workgroup on sepsis-associated mycoses in critically ill patients were therefore to identify new promising biomarkers within a comprehensive biomarker panel for the identification of septic shock patients suffering from an IFI as compared to colonized patients or patients without any fungal findings.

2. Material and Methods

2.1. Study Design. Data result from secondary analyses of an already published observational clinical study [21], which was approved by the local ethics committee (Ethics Committee of the Medical Faculty of Heidelberg, Trial Code No. S-097/2013/German Clinical Trials Register: DRKS00005463). The primary study was conducted in the surgical intensive care unit of Heidelberg University Hospital, Germany, between November 2013 and January 2015. All study patients or their legal designees gave written informed consent. In total, 50 patients suffering from septic shock according to the criteria of the Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock 2012 were enrolled in this study [25]. Treatment of patients with septic shock included early goal-directed therapy [26], elimination of the septic focus, and broad-spectrum antibiotics [26–28]. Blood samples were collected at septic shock onset (T0) and 1 day (T1), 2 days (T2), 7 days (T3), 14 days (T4), 21 days (T5), and 28 days (T6) thereafter. Relevant baseline data (demographic data, primary site of infection), clinical data (disease severity scores, such as Simplified Acute Physiology Score (SAPS) II, Sequential Organ Failure Assessment Score (SOFA), and Acute Physiology Health Evaluation score (APACHE) II), surgical procedures, antifungal therapy, and outcome parameters as well as routine infection parameters (e.g., leukocytes, C-reactive protein (CRP), procalcitonin (PCT), and body temperature) were collected. Moreover, EDTA plasma samples from 48 healthy individuals were obtained from Thermo Fisher Scientific internal serum bank, which served as quality controls (QC) for mass spectrometry (MS) measurements. These QC samples were pooled together before being divided into small aliquots, which were then stored along with the subjects' samples at −80°C until further use.

2.2. Mass Spectrometry. In total, 259 individual samples obtained from 50 included septic shock patients as well as pooled QC samples from 48 healthy individuals were processed following a protocol already described elsewhere [29]. Detailed information is also shown in the Supplementary Material 1.

2.3. Immunoassays. Plasma concentrations of procalcitonin (PCT) were measured with BRAHMS PCT-sensitive Kryptor (Thermo Fisher Scientific, BRAHMS GmbH, Hennigsdorf, Germany) on the Kryptor Compact Plus instrument. sICAM-1 immunoassay measurements were performed by ELISA on Platinum ELISA from eBioscience (Thermo Fisher Scientific, Waltham, USA).

2.4. Clinical Microbiology

2.4.1. Blood Culture. Blood culture testing at Heidelberg University Hospital was routinely performed as described elsewhere [30]. In brief, whole blood samples were obtained via direct venipuncture (e.g., antecubital vein) applying sterile techniques, and 10 mL blood was inoculated to both an aerobic and an anaerobic liquid culture medium (BACTEC PLUS, BD Biosciences, Heidelberg, Germany). Cultures were incubated for 5 days (BACTEC, BD Biosciences, Heidelberg, Germany) and positive cultures analyzed according to approved in-house hospital standard techniques, including identification by VITEK2 (bioMérieux, Marcy l'Etoile, France) or MALDI Biotyper (Bruker, Billerica, MA, USA) and automated antimicrobial susceptibility testing (VITEK 2).

2.4.2. Culture-Based Diagnostic Procedures in Tracheal Secretion, Wound Swabs, and Drainage Fluids. Briefly, tracheal aspirates and drainage fluids were streaked manually on Columbia (BD), chocolate (bioMérieux), MacConkey (bioMérieux), Schaedler and kanamycin-vancomycin (BD, Bi-plate), and chromogenic Candida agar (BD), while wound swabs were inoculated semiautomated by PREVI Isola™ instrument on the same agar types. All plates were incubated at 37°C in 5% CO_2 for 24 to 48 h, except the Schaedler-KV bi-plates, which were incubated at 37°C in an anaerobic

chamber (GasPak; Becton & Dickinson, Franklin Lakes, NJ) for 48 h as described elsewhere [31]. Bacterial and fungal colonies were identified by MALDI TOF mass spectrometry, and automated antimicrobial susceptibility testing was performed on VITEK 2 instruments (bioMérieux).

2.5. Group Definitions. *Candida* spp. in the respiratory tract or in fluids from drainages were classified as colonization. Positive results in blood cultures, intraoperative swabs, and *Aspergillus* spp. in deep respiratory tract specimens with accompanying pulmonary infiltrates were classified as infection.

2.6. Statistical Analyses. Ratio calculations after MS analysis were performed as described elsewhere [21]. All study data were entered into an electronic database (Excel 2013 Microsoft Corp, Redmond, USA) and evaluated using SPSS software (Version 24.0; SPSS, Inc., Chicago, USA). Principal Component Analysis (PCA) and correlations were performed with JUMP 13.1.0 software (SAS Institute, Cary, USA). Figures were created using GraphPad Prism (GraphPad Software, La Jolla, USA). Categorical data were summarized using absolute and relative frequencies. Quantitative data were summarized using median with quartiles. Mass spectrometry data are given as ratio without any units according to the mentioned protocol in Supplementary Material 1. The Kolmogorov-Smirnov test was applied to check for normal distribution. Due to nonnormally distributed data, nonparametric methods for evaluation were used (chi-square test for categorical data, Mann-Whitney U test for continuous data). The diagnostic performance of most suitable biomarkers (including biomarker combinations) for the identification of septic shock patients suffering from an IFI was assessed using a logistic regression model and/or receiver operating characteristic (ROC) analyses. A p value < 0.05 was considered statistically significant. Concerning symbolism and higher orders of significance: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

3. Results

3.1. Patients' Characteristics. Patients' characteristics are described in detail in Table 1. All patients suffered from bacterial septic shock. In total, 11 patients (22%) were shown to suffer from an additional IFI, whereas 22 patients (44%) were classified as being colonized. In contrast, 17 patients (34%) did not reveal any fungal findings within the entire study period. Occurrence of an IFI was shown to be associated with a significantly prolonged ICU stay as well as an increased need for mechanical ventilation. Disease severity scores did not differ significantly between the three study groups (Table 1).

3.2. MS-Based QC Measurements. Following a detailed preexisting protocol [29], QC measurements were performed. Results of the MS-based QC measurements are described in detail in Supplementary Material 2.

3.3. Panel-Based Biomarker Screening. At first, 62 plasmatic biomarkers were screened with regard to their diagnostic value for the prediction of an IFI in patients suffering from septic shock. Although 8 biomarkers revealed significant group differences at least at one or more timepoints (Supplementary Material Table 1), the following 3 biomarkers were shown to be the most promising for the detection of an IFI in septic shock patients.

Plasma levels of sICAM-1 were shown to be significantly increased within the first 7 days after the onset of septic shock in patients suffering from an IFI as compared to colonized patients or patients without any fungal findings (Figure 1(a)). Subsequently performed ROC analyses revealed promising performance characteristics of sICAM-1 with regard to the identification of an IFI in septic shock patients (Figure 1(b)). Concerning sICAM-1 at the timepoint of IFI diagnosis, plasma levels were significantly higher in the IFI group as compared to patients colonized with fungi (at the timepoint of the first fungal detection) or patients without any fungal findings (Figure 1(c)). A subsequently performed ROC analysis revealed an AUC of 0.707 for sICAM-1 at the timepoint of IFI diagnosis (Figure 2(a)).

Plasma levels of sICAM-1 did not differ significantly between surviving and nonsurviving patients and were not correlated with disease severity. Due to the bacterial origin of septic shock in all participating patients, the diagnostic value of sICAM-1 for the identification of patients suffering from bacterial septic shock cannot be assessed.

In parallel to MS-based measurements, plasma levels of sICAM-1 were also assessed by an immunoassay-based procedure for the timepoints T0 as well as T1. Comparable to the MS-based results with a Spearman correlation factor Rs = 0.89 (Supplementary Material Figure 1A), sICAM-1 was also significantly increased in septic shock patients suffering from an IFI as compared to colonized patients or patients without any fungal findings (Supplementary Material Figure 1B) using the immunoassay-based procedure. As assessed by an additional ROC analysis, immunoassay-based sICAM-1 measurements also proved suitable for early identification of septic shock patients suffering from an IFI (Supplementary Material Figure 1C).

Plasma levels of thrombospondin-1 were lower in patients with an IFI within the first 14 days after the onset of septic shock as compared to colonized patients or patients without any fungal findings. This difference was most pronounced between septic shock patients suffering from an IFI and those with a fungal colonization (Figure 3(a)). Subsequently performed ROC analyses revealed promising performance characteristics of thrombospondin-1 with regard to the identification of an IFI (Figure 3(b)). Concerning thrombospondin-1 at the timepoint of IFI diagnosis, plasma levels were lower in the IFI group as compared to fungal-colonized patients (at the timepoint of the first fungal finding) or patients without any fungal findings (Figure 3(c)). A subsequently performed ROC analysis revealed an AUC of 0.707 for thrombospondin-1 at the timepoint of IFI diagnosis (Figure 2(b)).

Plasma levels of vinculin were shown to be reduced in septic shock patients with an IFI as compared to the two other groups at several timepoints (Figure 4(a)). When comparing IFI patients with (a) patients without any fungal

Table 1: Patients' characteristics ($n = 50$).

	All patients ($n = 50$)	Without fungal isolates ($n = 17$)	Fungal colonization ($n = 22$)	Invasive fungal infection (IFI; $n = 11$)	p for patients without fungal infection vs. patients with fungal infection
Gender male	38 (76.0)	11 (64.7)	17 (77.3)	10 (90.1)	0.184
Age (years)	66 (61-75)	71 (64-80)	66 (61-74)	65 (58-74)	0.460
BMI (kg/m^2)	27.2 (24.4-30.9)	27.2 (25.7-34.9)	25.3 (21.6-30.8)	27.4 (26-30.5)	0.582
Postoperatively peritonitis initial operation	31 (62.0)	9 (52.9)	14 (63.6)	8 (72.7)	0.322
Kidney	2 (4.0)	0 (0.0)	1 (4.5)	1 (9.1)	0.395
Liver	11 (22.0)	1 (2.1)	3 (13.6)	7 (63.6)	**0.001*****
Pancreas	2 (10.0)	1 (5.9)	1 (4.5)	0 (0.0)	0.605
GIT	38 (76.0)	14 (82.4)	16 (72.7)	8 (72.7)	0.528
VAS	3 (6.0)	2 (11.8)	0 (0.0)	1 (9.1)	0.534
Others	12 (24.0)	3 (17.6)	5 (22.7)	4 (36.4)	0.240
≥48 h after hospital admission	25 (50.0)	7 (41.2)	15 (68.2)	3 (27.3)	0.085
NYHA 0-I	41 (82.0)	13 (76.4)	17 (77.3)	11 (100)	0.115
Diabetes mellitus	17 (34.0)	5 (29.4)	8 (36.4)	4 (36.4)	0.560
Arterial hypertension	34 (68.0)	12 (70.6)	15 (68.2)	7 (63.6)	0.495
Coronary heart disease	8 (16.0)	5 (29.4)	2 (9.1)	1 (9.1)	0.430
Chronic obstructive lung disease	10 (20.0)	5 (29.4)	5 (22.7)	0 (0.0)	0.062
Renal insufficiency	7 (14.0)	1 (5.9)	5 (22.7)	1 (9.1)	0.604
Renal replacement therapy	15 (30.0)	2 (11.8)	8 (36.4)	5 (45.5)	0.184
Liver cirrhosis	13 (26.0)	3 (17.6)	3 (13.6)	7 (63.6)	**0.003****
Oncological disease	33 (66.0)	11 (64.7)	14 (63.6)	8 (72.7)	0.440
APACHE II$^+$	30 (28-35)	32 (28-36)	30 (29-34)	29 (28-33)	0.335
SOFA$^+$	11 (10-14)	11 (10-14)	11 (10-13)	14 (11-15)	0.081
SAPS$^+$	65 (49-75)	72 (48-75)	61 (44-72)	68 (57-77)	0.519
Candida colonization	22 (44.0)	0 (0.0)	22 (100.0)	0 (0.0)	0.881
Candida infection	10 (20.0)	0 (0.0)	0 (0.0)	10 (90.1)	—
Candidemia	3 (6.0)	0 (0.0)	0 (0.0)	3 (27.3)	—
Aspergillus spp.	1 (3.0)	0 (0.0)	0 (0.0)	1 (9.1)	—
Candida score	4 (4-4)	4 (4-4)	4 (4-4)	4 (4-4)	—
Duration of mechanical ventilation (hours)	145.5 (67.3-450)	89 (46-145)	148.5 (74-239.3)	600 (424.5-944)	**0.007****
ICU length of stay (days)	19.5 (12-44)	12 (3-17)	21 (13.5-43.5)	38 (25.5-64)	**0.008****
Hospital length of stay (days)	44 (23.3-68.5)	24 (12-40)	50 (34.5-68.5)	53 (47.5-88)	0.075
Tracheotomy	14 (28.0)	2 (11.8)	2 (9.1)	8 (72.7)	**0.001*****
Anastomosis leakage	24 (48.0)	7 (41.2)	11 (50.0)	6 (54.5)	0.440
Fascia dehiscence	12 (24.0)	2 (11.8)	3 (13.6)	7 (63.6)	**0.002****
90-day mortality	17 (34.0)	8 (47.1)	4 (18.2)	5 (45.5)	0.293
28-day mortality	11 (22.0)	7 (41.2)	3 (13.6)	1 (9.1)	0.232
Gram-positive bacteria$^{\#}$	38 (76.0)	12 (70.6)	15 (68.2)	11 (100.0)	0.269
Enterococcus faecalis$^{\#}$	7 (14.0)	0 (0.0)	4 (18.2)	3 (27.3)	0.170
Enterococcus faecium$^{\#}$	22 (44.0)	5 (29.4)	10 (45.5)	7 (63.6)	0.127
Gram-negative bacteria$^{\#}$	30 (60.0)	8 (47.1)	14 (63.6)	8 (72.7)	0.269
Escherichia coli$^{\#}$	21 (42.0)	6 (35.3)	11 (50)	4 (36.4)	0.445
Pseudomonas aeruginosa$^{\#}$	10 (20.0)	2 (11.8)	3 (13.6)	5 (45.5)	**0.030***
Klebsiella pneumonia$^{\#}$	7 (14.0)	1 (5.9)	4 (18.2)	2 (18.2)	0.487

Legends: data are presented as count (rate) or median with accompanying quartiles. Abbreviations: BMI = body mass index; GIT = gastrointestinal tract; VAS = vascular artery surgery; NYHA = New York Heart Association score; APACHE II = Acute Physiology And Chronic Health Evaluation score; SAPS II = Simplified Acute Physiology Score; SOFA = Sequential Organ Failure Assessment Score; $^+$calculated at septic shock onset, $^{\#}$double-naming feasible, concerning symbolism and higher orders of significance: $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

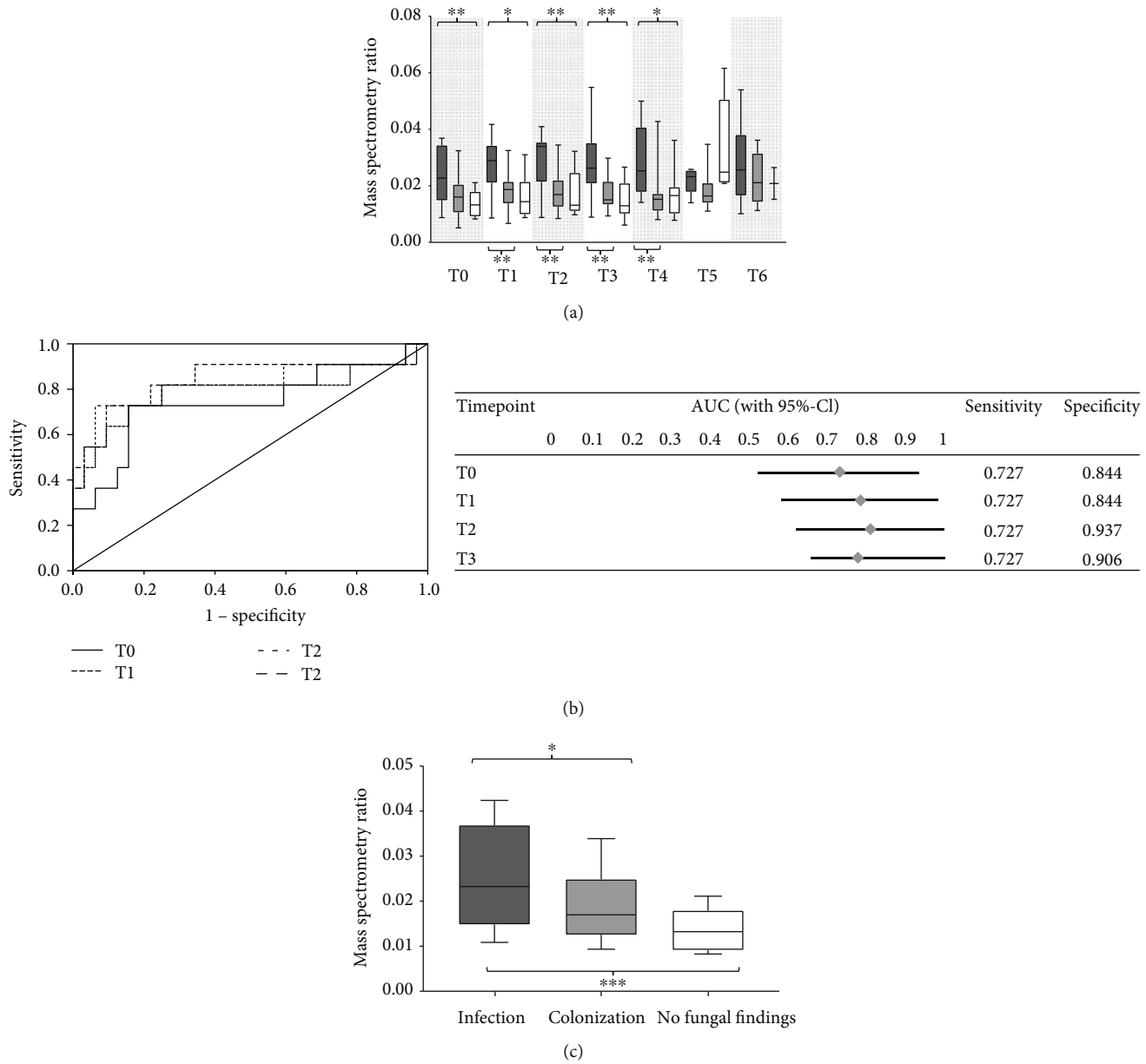

Figure 1: Plasma concentrations of sICAM-1 for the detection of an IFI in patients with septic shock. (a) Plasma concentrations of sICAM-1 were measured by mass spectrometry in patients suffering from septic shock with an invasive fungal infection (IFI, dark grey box), a fungal colonization (light grey box), or without any fungal findings (white box). Plasma samples were collected at the onset of septic shock (T0) and 1 day (T1), 2 days (T2), 7 days (T3), 14 days (T4), 21 days (T5), and 28 days (T6) afterwards. Data in box plots are given as median, 25th percentile, 75th percentile with the 10th as well as 90th percentile at the end of the whiskers. Concerning symbolism and higher orders of significance: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. (b) Receiver operating characteristic (ROC) analysis with sICAM-1 in all participating patients at septic shock onset (T0) and 1 day (T1), 2 days (T2), and 7 days (T3) afterwards with regard to the prediction of an invasive fungal infection (IFI) up to day 28. Patients suffering from an invasive fungal infection (IFI) represented the target group, whereas both patients with a fungal colonization and patients without any fungal isolates served as controls for this ROC analysis. Abbreviations: AUC: area under the curve; CI: confidence interval. (c) Plasma concentrations of sICAM-1 were measured by mass spectrometry in patients suffering from septic shock with an invasive fungal infection (IFI, dark grey box), a fungal colonization (light grey box), or without any fungal findings (white box). In IFI patients as well as in those with a fungal colonization, plasma concentrations of sICAM-1 are presented for the timepoint of first fungal detection in microbiological samples. In patients with no fungal findings, plasma concentrations of sICAM-1 at septic shock onset are presented. Data in box plots are given as median, 25th percentile, 75th percentile with the 10th as well as 90th percentile at the end of the whiskers. Concerning symbolism and higher orders of significance: $^{*}p < 0.05$, $^{***}p < 0.001$.

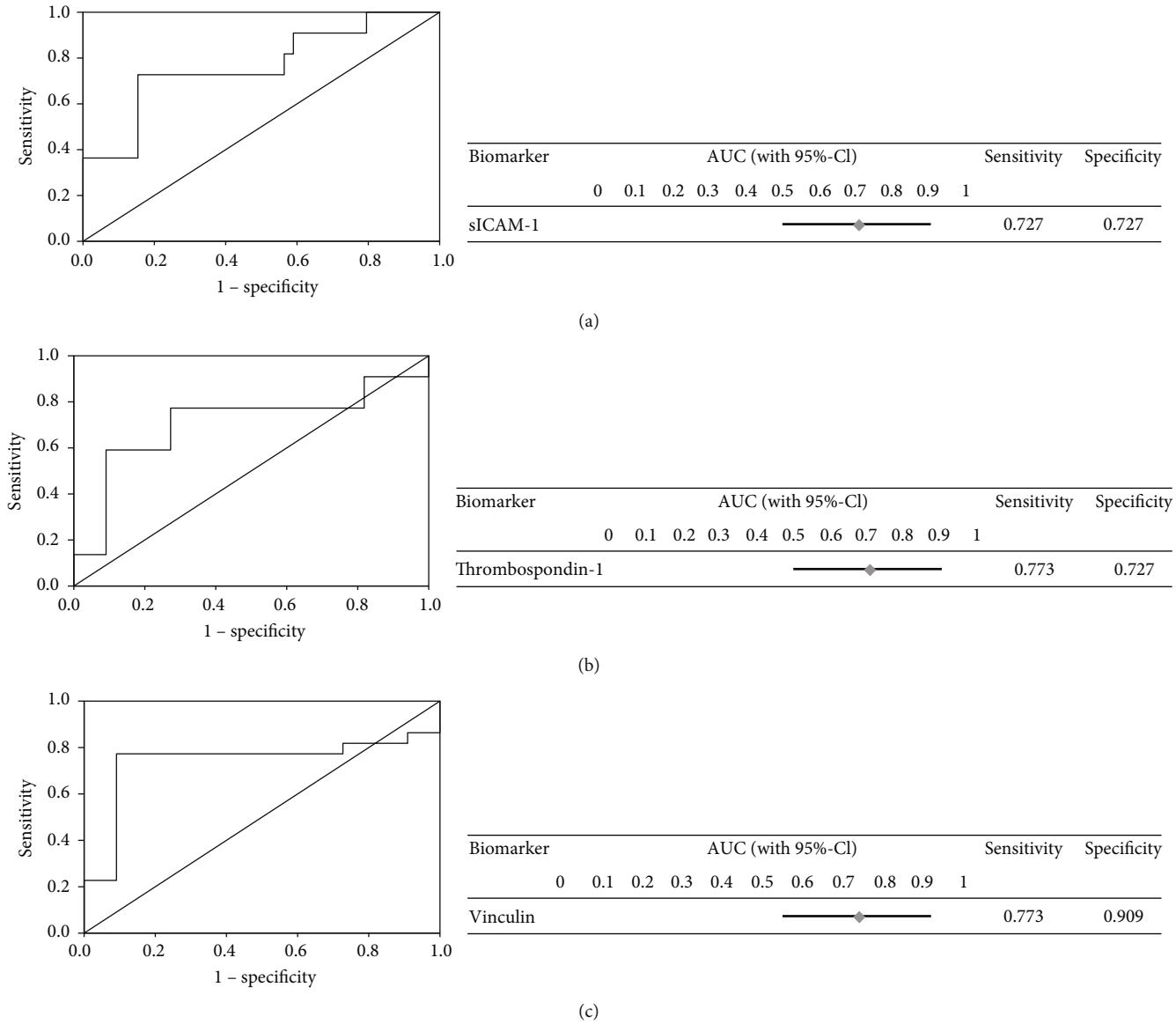

Figure 2: Receiver operating characteristic (ROC) analyses for sICAM-1, thrombospondin-1, and vinculin for IFI diagnosis at first detection of fungal pathogens. (a) ROC analysis for sICAM-1 in fungal-infected vs. fungal-colonized patients at first detection of fungal pathogens. Abbreviations: AUC: area under the curve; CI: confidence interval. (b) ROC analysis for thrombospondin-1 in fungal-infected vs. fungal-colonized patients at first detection of fungal pathogens. Abbreviations: AUC: area under the curve; CI: confidence interval. (c) ROC analysis for vinculin in fungal-infected vs. fungal-colonized patients at first detection of fungal pathogens. Abbreviations: AUC: area under the curve; CI: confidence interval.

findings, this effect was most pronounced at T1, T2, and T4, whereas (b) patients with a fungal colonization only differed significantly at T4. Subsequently performed ROC analyses revealed promising performance characteristics of vinculin with regard to the identification of an IFI (Figure 4(b)). Concerning vinculin at the timepoint of IFI diagnosis, plasma levels were lower in the IFI group as compared to fungal-colonized patients (at the timepoint of the first fungal finding) or patients without any fungal findings (Figure 4(c)). A subsequently performed ROC analysis revealed an AUC of 0.740 for vinculin at the timepoint of IFI diagnosis (Figure 2(c)).

Using a logistic regression model, the combined use of sICAM-1, thrombospondin, and vinculin was shown to result in an additional value for IFI diagnosis, with the best AUC of 0.921 at 7 days following the onset of septic shock (T3) (Supplementary Material Figure 2).

As previously described throughout our workgroup, the diagnostic performance of procalcitonin (PCT), BDG, and CRP for early IFI detection within the presented cohort of septic shock patients was poor [21]. However, the combination of these routinely used infection biomarkers (e.g., PCT) and innovative fungal biomarkers (e.g., MR-proADM, sICAM-1, and IL-17A) was shown to improve the

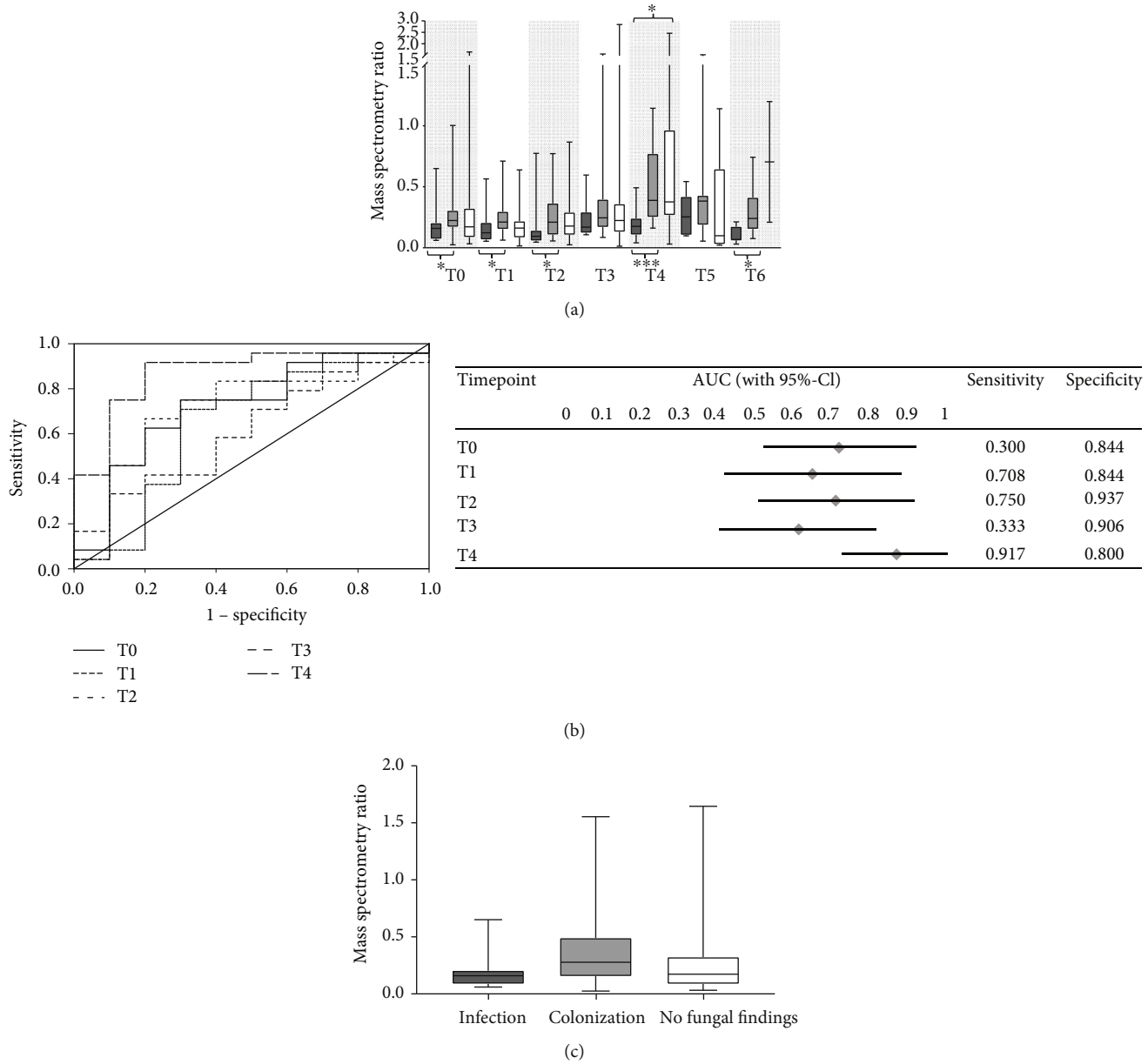

Figure 3: Plasma concentrations of thromospondin-1 for the detection of an IFI in patients with septic shock. (a) Plasma concentrations of thrombospondin-1 were measured by mass spectrometry in patients suffering from septic shock with an invasive fungal infection (IFI, dark grey box), a fungal colonization (light grey box), or without any fungal findings (white box). Plasma samples were collected at the onset of septic shock (T0) and 1 day (T1), 2 days (T2), 7 days (T3), 14 days (T4), 21 days (T5), and 28 days (T6) afterwards. Data in box plots are given as median, 25th percentile, 75th percentile with the 10th as well as 90th percentile at the end of the whiskers. Concerning symbolism and higher orders of significance: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. (b) Receiver operating characteristic (ROC) analysis with thrombospondin-1 in all participating patients at septic shock onset (T0) and 1 day (T1), 2 days (T2), 7 days (T3), and 14 days (T4) afterwards with regard to the prediction of an invasive fungal infection (IFI) up to day 28. Patients suffering from an invasive fungal infection (IFI) represented the target group, whereas both patients with a fungal colonization and patients without any fungal isolates served as controls for this ROC analysis. Abbreviations: AUC: area under the curve; CI: confidence interval. (c) Plasma concentrations of thrombospondin-1 were measured by mass spectrometry in patients suffering from septic shock with an invasive fungal infection (IFI, dark grey box), a fungal colonization (light grey box), or without any fungal findings (white box). In IFI patients as well as in those with a fungal colonization, plasma concentrations of thrombospondin-1 are presented for the timepoint of first fungal detection in microbiological samples. In patients with no fungal findings, plasma concentrations of thrombospondin-1 at septic shock onset are presented. Data in box plots are given as median, 25th percentile, 75th percentile with the 10th as well as 90th percentile at the end of the whiskers. Concerning symbolism and higher orders of significance: $^{*}p < 0.05$, $^{***}p < 0.001$.

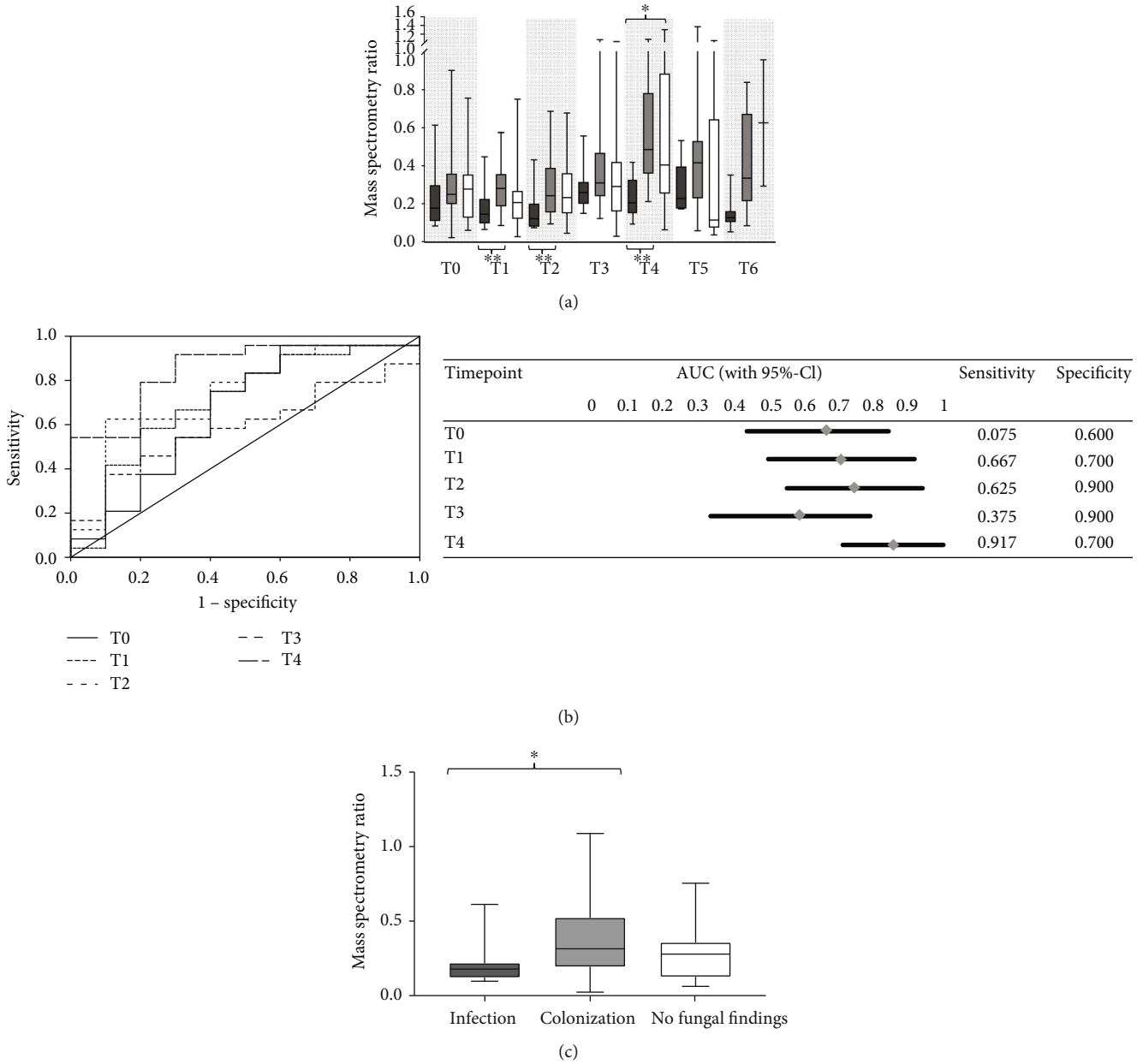

Figure 4: Plasma concentrations of vinculin for the detection of an IFI in patients with septic shock. (a) Plasma concentrations of vinculin were measured by mass spectrometry in patients suffering from septic shock with an invasive fungal infection (IFI, dark grey box), a fungal colonization (light grey box), or without any fungal findings (white box). Plasma samples were collected at the onset of septic shock (T0) and 1 day (T1), 2 days (T2), 7 days (T3), 14 days (T4), 21 days (T5), and 28 days (T6) afterwards. Data in box plots are given as median, 25th percentile, 75th percentile with the 10th as well as 90th percentile at the end of the whiskers. Concerning symbolism and higher orders of significance: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. (b) Receiver operating characteristic (ROC) analysis with vinculin in all participating patients at septic shock onset (T0) and 1 day (T1), 2 days (T2), 7 days (T3), and 14 days (T4) afterwards with regard to the prediction of an invasive fungal infection (IFI) up to day 28. Patients suffering from an invasive fungal infection (IFI) represented the target group, whereas both patients with a fungal colonization and patients without any fungal isolates served as controls for this ROC analysis. Abbreviations: AUC: area under the curve; CI: confidence interval. (c) Plasma concentrations of vinculin were measured by mass spectrometry in patients suffering from septic shock with an invasive fungal infection (IFI, dark grey box), a fungal colonization (light grey box), or without any fungal findings (white box). In IFI patients as well as in those with a fungal colonization, plasma concentrations of vinculin are presented for the timepoint of first fungal detection in microbiological samples. In patients with no fungal findings, plasma concentrations of vinculin at septic shock onset are presented. Data in box plots are given as median, 25th percentile, 75th percentile with the 10th as well as 90th percentile at the end of the whiskers. Concerning symbolism and higher orders of significance: $^{*}p < 0.05$, $^{***}p < 0.001$.

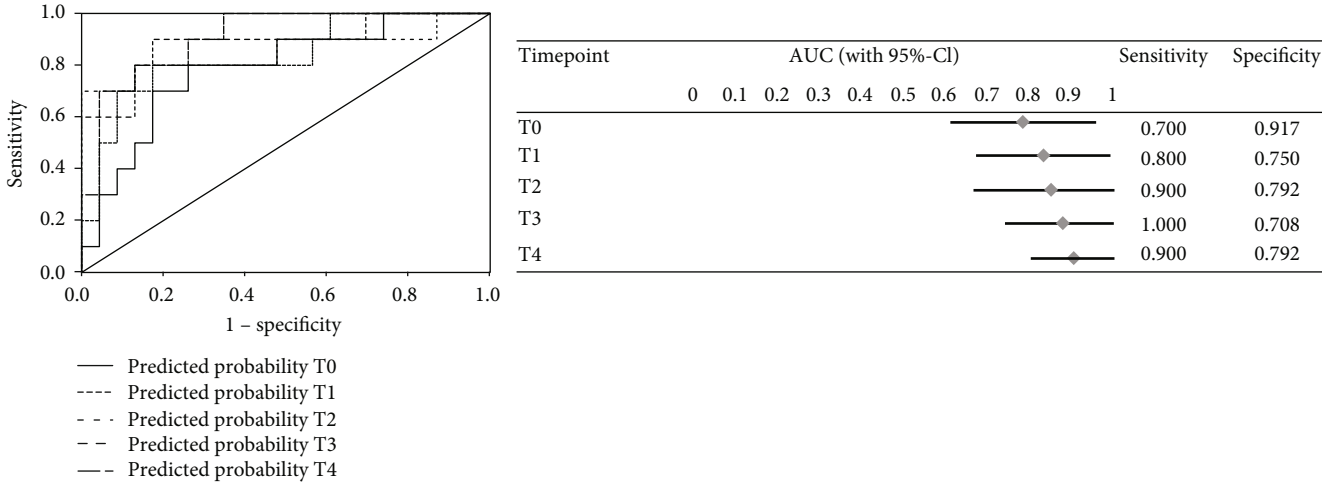

Figure 5: ROC analyses for combined measurements of MR-proADM and sICAM-1 for the detection of an IFI in patients with septic shock. Receiver operating characteristic (ROC) analyses with MR-proADM and sICAM-1 in all participating patients at septic shock onset (T0), day 1 (T1), day 2 (T2), day 7 (T3), and day 14 (T4) afterwards with regard to the prediction of an invasive fungal infection (IFI) up to day 28. Patients suffering from an invasive fungal infection (IFI) represented the target group, whereas both patients with a fungal colonization and patients without any fungal isolates served as controls for this ROC analysis. Abbreviations: AUC: area under the curve; CI: confidence interval.

diagnostic performance (Supplementary Material Table 2). In particular, the combination of MR-proADM with sICAM-1 revealed the most promising results with the best AUC of 0.909 at 14 days after the onset of septic shock (T4) (Figure 5).

4. Discussion

This secondary analysis of prospectively collected data identified sICAM-1, thrombospondin-1, and vinculin as suitable biomarkers for early identification of septic shock patients suffering from an IFI as compared to solely colonized patients or patients without any fungal findings. The diagnostic value could be further strengthened by a combination of the aforementioned biomarkers as well as the combined use of sICAM-1 and MR-proADM (which was previously reported to be of diagnostic value in sepsis-associated IFI [21]).

The diagnosis of an IFI remains a challenge in daily clinical routine, as most of the available diagnostic tools are associated with relevant weaknesses [20, 21]. In particular, the sensitivity of culture-based technologies is known to be poor, so that only a small portion of septic patients with an IFI can be identified in the early disease course [32, 33]. Therefore, Leon et al. introduced the so-called "Candida score" in 2006 in order to predict the risk for a fungal infection in nonneutropenic critically ill patients, consisting of parenteral nutrition, surgery, multifocal colonization, and severe sepsis [34]. Nevertheless, therapy guidance by the use of this score may lead to an unnecessary overtreatment, as most of critically ill patients will achieve a high "Candida score" without suffering from an IFI [21]. This is of great relevance, since an antimycotic overtreatment is associated with several problems, potentially affecting patient's course of the disease: (1) antimycotics do have relevant undesired side effects and may harm the critically ill patient [35] and (2) an inadequate use of anti-infective drugs is always associated with an increase in resistance mechanisms [36]. Although new diagnostic approaches such as PCR- or NGS-based methods might be able to overcome the aforementioned weaknesses of culture-based methods, they have not been implemented into clinical routine up to now [21, 24]. Therefore, diagnosis of an IFI by direct (culture-based) or indirect (NGS- or PCR-based) detection of the causing pathogen in combination with a risk factor-based "Candida scoring system" is far from perfect. Therefore, additional plasmatic biomarkers might be of great help for the clinician in order to close the diagnostic gap and to guide antimycotic therapy in IFI-affected patients, potentially leading to a significant reduction of morbidity and mortality as well as a more specific antifungal drug use. This will hopefully be associated with the following most appreciated side effects: (1) primary cost savings (e.g., due to the waiving of expensive antifungal drugs and reduced hospital and ICU stay) and (2) reducing drug resistance rates (with secondary cost savings). Although the combined use of BDG and PCT was recently described to be suitable for early differential diagnosis between candidemia and bacteremia in intensive care units [22], the diagnostic value of BDG for IFI diagnosis is not free of doubts and subject of controversial discussions [37–40]. In line with that, BDG recently failed to be a suitable biomarker for reliable IFI diagnosis in septic shock within the presented cohort [21]. Therefore, a comprehensive screening of 62 potentially suitable new biomarkers has now been performed by targeted MS, where the following three plasmatic biomarkers were shown to be most suitable.

ICAM-1 is a 58 kDa single-chain protein expressed on the cell surface of, e.g., B-lymphocytes [41], which has

regulatory effects in inflammation processes and acts as a ligand for the lymphocyte-associated antigen- (LFA-) 1 [42]. In infections with *C. albicans* or *A. fumigatus*, ICAM-1 is known to be upregulated and seems to play an important role in the host defense against these pathogens [43]. An inhibition of ICAM-1 leads to a reduced adherence of *C. albicans* to human gingival epithelial cells and thus to a decreased secretion of IL-8, which acts as a proinflammatory molecule in fungal infections [44–46]. Following proteolytic cleavage from the cell membrane, a soluble isoform entitled sICAM-1 can be detected in several fluids, including plasma [47, 48]. Besides fungal infections, sICAM-1 has also been described to be of relevance in many other diseases such as cancer, autoimmune diseases, or bacterial sepsis and was shown to serve as a marker for disease severity [49–52]. To sum up, sICAM-1 has an important role in the host defense against fungal infections, which can be supported by plasmatic concentration profiles of sICAM-1 within the presented investigation. Accordingly, plasma levels of sICAM-1 were significantly increased in septic shock patients suffering from an IFI within the first 14 days after septic shock onset as compared to fungal-colonized patients or patients without any fungal findings. An elevation of sICAM-1 plasma levels after septic shock onset might therefore represent an early risk assessment tool for an emerging IFI in septic shock patients. Accordingly, this might aid clinicians to decide for or against an antimycotic therapy especially in those patients, where routinely used techniques for the diagnosis of an IFI fail.

Thrombospondin-1 is a 129 kDa glycoprotein of the extracellular matrix with a variety of functions. Thrombospondin-1 is secreted from different cancer types [53], after tissue damage as well as in various inflammatory settings [54, 55], resulting in the activation of proinflammatory macrophages [56]. Moreover, thrombospondin-1 has previously been described to play an important role in the pathogenesis of several infectious disease states, e.g., induced by *Streptococcus pneumonia* or *Staphylococcus* spp. [57, 58]. Although these publications describe a close connection between thrombospondin-1 plasma levels and solely gram-positive infections, the same seems to hold true for other infection types since the presented cohort of septic shock patients mainly suffered from polymicrobial postoperative peritonitis, including gram-positive as well as gram-negative pathogens (Table 1). With regard to infections caused by *C. albicans*, thrombospondin-1 was shown to aggravate the inflammatory response by inhibiting fungal phagocytic clearance within a mice model [59]. Within the presented investigation of human septic shock patients, thrombospondin-1 was shown to be decreased in septic shock patients suffering from an additional IFI as compared to fungal colonized patients or patients without any fungal findings. In line with the aforementioned findings of Martin-Manso et al. [59], this might indicate an increased phagocytosis capacity of *C. albicans* due to reduced thrombospondin-1 levels, therefore representing an effective defense mechanism of the immune system to enhance fungal phagocytosis. Within daily clinical routine, thrombospondin-1 levels might be of additional help for the differentiation of a fungal colonization from an IFI in case of positive fungal microbiological cultures apart from bloodstream infections.

Vinculin is a 116 to 124 kDa protein which is responsible for the linkage of adhesion molecules to the actin cytoskeleton [60]. In its inactive isoform, it is located in the cytoplasm, whereas the active phenotype (regulated by a C-terminal tail domain) is located on the cell surface [61, 62]. It is described to be of relevance in patients with gastric cancer [63] and plays an important role in the uptake of bacterial pathogens, e.g., *S. aureus* [64]. Although Hagiwara et al. [64] describe a close connection between plasma levels of vinculin and gram-positive infections caused by *S. aureus*, the same seems to hold true for other infectious disease entities. Accordingly, Thwaites et al. were able to demonstrate a vinculin-mediated interaction to facilitate pathogen invasion in the host cell in infections caused by *Chlamydia* spp. [64, 65]. Although the extent of vinculin release in septic patients seems to be closely connected to the presence of bacterial pathogens, its role in fungal infections has not been reported yet. However, within the presented investigation, plasma levels of vinculin were shown to be the lowest in patients suffering from an IFI as compared to the two other groups, potentially indicating an immunosuppressive disease state with a high risk for the development of an IFI. Although the exact role of vinculin remains somewhat unclear, decreased vinculin plasma levels were shown to be suitable for the identification of septic shock patients at high risk for the development of an IFI in the course of the disease.

Apart from decision-making based on single biomarkers, measurements of a representative biomarker panel (consisting of the most helpful IFI biomarkers) might be of additional diagnostic value. Accordingly, combined measurements of standard infection markers (e.g., PCT) with innovative fungal biomarkers (e.g., MR-proADM, sICAM-1, and IL-17A) resulted in an improved diagnostic performance for the identification of an IFI in human septic shock. However, the combination of MR-proADM with sICAM-1 was shown to be the most suitable.

5. Limitations

Although the results of our secondary analysis appear to be sound and conclusive, the following limitations need to be addressed in connection with the presented manuscript. The clinical investigation was performed in terms of an observational single-centre study and is therefore characterized by a small number of participating patients, representing a highly selective cohort of critically ill patients suffering from septic shock with or without an IFI.

6. Conclusions

Plasmatic measurements of sICAM-1, thrombospondin-1, and vinculin (or a combination thereof) might be able to facilitate the diagnosis of an IFI in patients suffering from septic shock. Moreover, these markers may be used for the identification of patients at high risk for the development of an IFI already at septic shock onset or shortly thereafter. This may help clinicians to decide for or against an antimycotic

therapy especially in those patients, where routinely used techniques for the diagnosis of an IFI fail. Nevertheless, due to the methodological limitations of the presented work that are described, additional clinical investigations need to be recommended in order to definitely determine the diagnostic value of sICAM-1, thrombospondin-1, and vinculin for the identification of patients suffering from an IFI.

Data Availability

The raw data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

SOD conceived the study, participated in its design and coordination, and drafted the article. Furthermore, he performed data acquisition and prepared the tables and figures. AS performed data acquisition and was involved in critical revision of the manuscript. AI and CH were responsible for laboratory measurements and were involved in critical revision of the manuscript; MAW and FU participated in the design of the study and were involved in critical revision of the article. TBru participated in the design of the study and performed statistical analyses. Furthermore, he was involved in revising the manuscript critically. AH and SZ were responsible for microbiological analyses and were involved in revising the manuscript critically. TBre conceived the study, participated in its design, coordinated, and helped to draft the article. All authors read and approved the final manuscript.

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Supplementary Materials

Supplementary Material 1: description of mass spectrometry method. Supplementary Material 2: description of mass spectrometry quality control. Supplementary Material Table 1: examples of screened proteins. Supplementary Material Table 2: receiver operating curve (ROC) analyses for different biomarker combinations. Supplementary Material Figure 1: immunoassay-based measurements of plasmatic sICAM-1 concentrations for the detection of an IFI in patients with septic shock. Supplementary Material Figure 2: ROC analyses for combined measurements of sICAM-1, thrombospondin-1, and vinculin for the detection of an IFI in patients with septic shock. Supplementary Material 3: STROBE Statement—checklist of items that should be included in reports of observational studies. *(Supplementary Materials)*

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