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Prognostic value of serum galactomannan in mixed ICU patients: a retrospective observational study

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Abstract

Background: Little is known about serum galactomannan (GM) testing in (mostly non-neutropenic) mixed intensive care unit (ICU) patients. The aim of this study was to look for the incidence of invasive aspergillosis (IA) in critically ill patients, to validate previously reported GM thresholds, and to evaluate the prognostic value of GM.

Methods: This was a retrospective study of 474 GM samples in 160 patients from the start of January 2003 until the start of February 2004. GM tests were ordered because of a clinical suspicion of IA or on a regular basis in immune compromised patients. The number of samples per patient was 3 ± 2.6. We used the criteria of the European Organisation for Research and Treatment of Cancer (EORTC) to define proven IA, probable IA, and possible IA. The number of positive samples, with GM optical density (OD) > 0.5 was 230 (48.5%).

Results: In our study population, five (3%) patients had proven IA, 11 (7%) had probable, 27 (17.5%) had possible, and 116 (72.5%) had no IA. We could not identify a GM threshold for IA with analysis of receiver operating characteristics (ROC) curves: with a sensitivity of (56.3%, 50%, 50%, 37.5%), specificity (38.2%, 67.5%, 68.8%, 72.9%), NPV (88.7%, 91.8%, 92.5%, 91.3%) and PPV (9.2%, 12.9%, 15.1%, 13.3%) for a cut-off of OD > 0.5, > 0.8, > 1.1 and > 1.5 respectively. IA was associated with high mortality of 87.5% and 100% in patients with probable and proven IA respectively. Patients with IA had a significant increase of GM during their stay (GM_{delta} 0.7 ± 1.5 vs –0.2 ± 1.5, $P = 0.027$). The overall ICU mortality was 41.9% and the hospital mortality was 58.1%. Patients who died in the ICU and in the hospital had higher APACHE-II, SAPS-II and SOFA scores ($P < 0.0001$) and also a significant increase in GM during their stay with 0.27 ± 1.26 (ICU non-survivors) and 0.11 ± 1.55 (hospital non-survivors) compared to a decrease in GM –0.43 ± 1.7 ($P = 0.004$) and –0.48 ± 1.51 ($P = 0.017$) in ICU and hospital survivors respectively. Non-survivors also had higher mean GM values but this was not statistically significant. There was a trend towards higher GM values in patients treated with piperacillin/tazobactam (n = 34), but this did not reach statistical significance. Neutropenic patients (n = 31) showed an increase in GM during their stay 0.32 ± 1.3 vs a decrease with –0.43 ± 1.7 in non-neutropenic patients ($P = 0.07$). Patients on total parenteral nutrition (n = 125) had higher maximal GM levels (1.55 ± 1.94 vs 0.88 ± 1.25, $P = 0.058$). Patients who were mechanically ventilated had significantly higher mean ($P = 0.038$) and maximal ($P = 0.007$) GM levels.

Conclusions: We found a high incidence of proven and probable IA in a group of mixed ICU patients (10%) and the presence of IA was associated with a high mortality. The serum GM antigen detection test may not be

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useful in the diagnosis of IA in mixed ICU patients, according to the results of the ROC analysis. We could not define a useful threshold.

Key words: critical care, invasive aspergillosis, serum galactomannan, mixed ICU patients, non-neutropenic, predictive value, prognosis

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Invasive aspergillosis (IA) remains a frequently missed diagnosis in the ICU, which is shown by studies that compare the premortem diagnosis with autopsy. Nine cases of IA out of 149 were missed in a surgical ICU [1]. Another single centre study showed 15 cases of IA out of 100 autopsies, of which five cases were missed [2], and Valles et al. found IA in 13 out of 69 patients in the ICU with hospital-acquired pneumonia [3]. The incidence of IA in the mixed ICU population is unclear and varies between 0.33% and 6.9% [4]. In a retrospective autopsy-controlled study, the majority of patients with IA (70%) did not have a classical risk factor of IA such as a haematological malignancy or allogenic stemcell transplantation. New risk factors that predispose to IA in critically ill patients are chronic obstructive pulmonary disease (COPD), liver cirrhosis, prolonged therapy with corticosteroids, and severe sepsis. But the European Organisation for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) guidelines are designed only for patients with cancer or patients after bone marrow transplantation. It is more difficult to diagnose IA in mixed ICU patients compared to neutropenic patients and the criteria that are used are not well studied in non-neutropenic mixed ICU patients. First, the gold standard for the diagnosis of proven IA is thoracoscopic or open lung biopsy, which is difficult in ICU patients who often have coagulopathy and haemodynamic instability. Second, patients in ICU often don't have the classical host factor which is necessary to diagnose probable or possible IA, and third, radiological findings are often aspecific in patients who are mechanically ventilated.

Because of the high mortality in patients with IA and the difficulty of diagnosing IA in ICU patients, it would be interesting to establish an early diagnosis with an alternative test or diagnostic algorithm. Serum aspergillus antigen or Galactomannan (GM) has been reported as a reliable test for detecting IA in neutropenic patients with underlying haematological malignancies [5]. By using GM, the diagnosis of IA can be established eight days before a diagnosis can be made by other investigations [6]. Serum GM is a polysaccharide fungal-cell wall component that is released during tissue invasion by Aspergillus hyphae. This component can be detected by a sandwich enzyme linked immunosorbent assay (ELISA, Platelia Aspergillus, Bio-Rad, France). But little is known about GM testing in mostly non-neutropenic mixed ICU patients. In one retrospective study, the GM was positive in only 53% of the patients with IA [7], and in 2007 the same investigators found a sensitivity of only 42% of serum GM in non-neutropenic patients [8].

Recent studies have confirmed the use of GM as a possible outcome measure for IA, based on a strong correlation between increased GM levels and poor clinical outcomes in haematologic malignancies [9, 10]. To date, no study has confirmed this association in non-haematologic patients. The aim of this study therefore was first to establish the incidence of IA in mixed ICU patients, second to validate previously reported GM thresholds, and third to evaluate the prognostic value of GM in critically ill patients.

METHODS

DATA COLLECTION

Using the electronic ICU patient database, patient demographics, GM levels, sputum cultures, biopsy and autopsy findings were collected. Severity of illness was evaluated using the Simplified Acute Physiology Score (version 2; SAPS-II) and the Acute Physiology and Chronic Health Evaluation (version 2; APACHE-II). Patient data was accessed via the database program (FileMaker Pro and Deio Clinisoft) and exported to an Excel worksheet (Microsoft, Redmond, WA, USA). All adult patients (> 18 years old) admitted to the ICU who stayed longer than five days or whose lungs were mechanically ventilated were included.

GM TEST

In our hospital, GM analysis is done using Platelia (Bio-Rad, France). This is a single-stage immune-enzymatic sandwich microplate assay that uses rat monoclonal antibody (EBA-2) to detect circulating GM antigen, in this case in serum. The GM tests were ordered because of a clinical suspicion of IA or on a regular basis in immune compromised patients. According to the best threshold previously reported in neutropenic patients, our threshold to define a positive GM was an optical density (OD) > 0.5. The first GM (GM_{first}) is the first sample taken in each patient. The maximal GM (GM_{max}) is maximal GM level during ICU stay and the delta GM (GM_{delta}) is the difference between the first GM sample and the maximal GM level.

DEFINITIONS

Similarly to the 2008 EORTC criteria, we defined proven IA, probable IA, possible IA and no IA, although with the modification that we used a broader spectrum of the host factor criteria, including COPD, liver cirrhosis and patients with solid organ transplantation. Detection of GM in serum was not included as a microbiological criterion. *Proven IA* referred to the histopathologic evidence of tissue invasion by septated, acutely branching filamentous fungi. *Probable IA* referred to the presence of a positive culture or cytology for Aspergillus species together with one clinical criterion (symptoms of lower respiratory tract infection, tracheobronchitis, symptoms of infection of the paranasal sinuses, or symptoms of infection of the central nerve system). For *possible IA* we used clinical criteria but without mycological support. We defined patients in the 'proven' and 'probable' groups as positive IA.

STATISTICAL ANALYSIS

Continuous variables are presented as either mean (± SD) or median with interquartile range in case of skewed distribution; categorical variables are expressed as numbers and percentages of the group from which they were derived. All comparisons were unpaired. Continuous variables were compared with the Student's *t*-test for normally distributed variables and the Mann Whitney U test for non-normally distributed variables. The χ^2 test and Fisher's exact test were used to compare categorical variables. Odds ratios (ORs) are given with 95% confidence intervals (CIs). For the determination of the best predictive cut-offs, receiver operating characteristics (ROC) curves were constructed for GM. ROC-curves help us to evaluate a test for its overall discriminatory power, to predict for instance survival from non-survival and the presence of IA from the absence of IA. ROC curves graph the sensitivity of a diagnostic test (true positive portion) versus 1 minus the specificity (false positive portion). A test with absolute discriminatory power i.e. that always predicts the presence of IA or no IA and survival or non--survival has an area under the ROC curve of 1, and a test that predicts survival no more often than by chance has an area under the ROC curve of 0.5. In general, the closer the ROC curve follows the left upper corner, the more accurate the test and the closer the curve comes to the 45° reference line, the less accurate. Analysis of the co-ordinates of the curve can then identify the cut-off with the best sensitivity and specificity. The relationship between continuous variables was assessed by means of Pearson's linear correlation coefficient. Statistical analysis was performed with SAS (version 8.2) and SPSS (Windows version 17.1, Chicago, IL, USA). All *P* values were two-tailed, and a *P* lower than 0.05 was considered statistically significant.

ETHICAL CONSIDERATIONS

This study was conducted in accordance with the ICU protocol, the Declaration of Helsinki and applicable regulatory requirements as approved by the institutional review board and the local institutional ethics committee. This study was a pilot study, performed in anticipation of a prospective epidemiological study (EC approval number 2411, 8 August 2004). In view of the nature of the study being purely observational and not demanding a deviation from standard clinical ICU care, informed consent from the patient or the next of kin was waived. We merely analysed retrospectively the existing situation and did nothing to influence events. Only the treating ICU physicians accessed the medical records. All data was pseudonymised before analysis. This is a single centre retrospective cohort study conducted on the electronic files of the patients admitted to the mixed 12-bed medical and surgical ICUs of a tertiary hospital (Ziekenhuis Netwerk Antwerpen, ZNA Stuivenberg, Antwerp, Belgium) during a 13-month period.

RESULTS

PATIENT CHARACTERISTICS

Between 1 January 2003 and 1 February 2004, 474 GM samples were taken in a mixed ICU population of 160 patients (80 men and 80 women with a mean age of 64.5 ± 15.9 years). The number of samples per patient was 3 ± 2.6. The characteristics of the 16 patients with IA are shown in Table 1. In our study population, five (3%) had proven IA, 11 (7%) had probable, 28 (17.5%) had possible, and 116 (72.5%) had no IA. In the group of patients with proven and probable IA, there were four patients (25%) with a haematologic disease. Nine patients (56%) had chronic obstructive pulmonary disease (COPD) and steroid use. Further, there was one patient with Goodpasture disease, one patient with alcoholic liver disease, and one patient who had received a heart transplantation in the past. One third of the patients were neutropenic. All of the neutropenic patients in the IA group had a positive GM. Autopsy was only performed in three out of five patients with proven IA.

Table 2 shows no significant difference in the first, maximal or mean GM levels between survivors and non-survivors and between patients with or without IA. There is only a significant difference in GM_{delta} levels, which suggests an elevation of GM during the ICU stay in patients with IA and in ICU and hospital (HOS) non-survivors ($P = 0.027$, $P = 0.004$ and $P = 0.017$ respectively) (Fig. 1). Patients with IA had a longer duration of mechanical ventilation, but this was not statistically significant ($P = 0.96$).

TEST CHARACTERISTICS

Of the 474 GM samples, 230 GM samples were positive (48.5%) with a mean value of 0.94 OD (Fig. 2). In 16 patients

Table 1. Patient characteristics of cases with proven and probable IA

Patient No	EORTC	Age	Gender	LOS	Admission diagnosis	Host factor	APACHE	SAPS	SOFA	Neutro-penia	outcome	Antifungal Therapy	Culture/ BAL	n	GM_{max} ± SD	GM_{mean}	APO
1	1	61	F	36	Encephalopathy	Lymphoma	30	68	7	yes	Death	Caspo+Vorico	neg	9	2.6	1.58 ± 0.8	Biopsy
2	1	57	M	16	Pneumonia	Liver cirrhosis	12	34	5	no	Death	No	neg	2	0.1	0.1 ± 0	Autopsy
3	1	66	F	39	Pneumonia	Bone marrow Tx	33	74	9	yes	Survived	Caspo	neg	7	0.5	0.5 ± 0	Biopsy
4	1	83	M	26	COPD	COPD, steroids	11	46	2	no	Survived	Ampho	pos	6	1.23	0.42 ± 0.4	Autopsy
5	1	57	F	13	Acute Kidney Injury	Goodpasture	21	43	5	yes	Death	Ampho	pos	2	5.2	3 ± 2.3	Autopsy
6	2	84	M	15	Pneumonia	COPD, steroids	16	51	9	no	Death	Ampho	pos	3	0.40	0.35 ± 0.1	
7	2	69	M	14	COPD	COPD, steroids	16	34	3	no	Death	Ampho	pos	1	0.03	0.03	
8	2	59	M	4	COPD	COPD, steroids	21	34	5	no	Death	Caspo+Vorico	pos	3	5	3.9 ± 1.9	
9	2	72	F	18	Pneumonia	COPD, steroids	30	55	7	no	Death	Ampho	pos	4	0.1	0.1 ± 0	
10	2	74	M	4	Pneumonia	AML	23	46	10	yes	Death	No	pos	1	1.50	1.5	
11	2	76	M	5	Sepsis	Haematologic malignancy	24	71	7	yes	Death	Caspo	pos	2	4.10	4.1 ± 0	
12	2	70	M	16	COPD	COPD, steroids	12	38	0	no	Survived	No	pos	3	0.20	0.13 ± 0	
13	2	65	M	4	Pneumonia	COPD, steroids	6	9	0	no	Survived	Caspo+Vorico	pos	1	0.15	0.15	
14	2	77	M	25	Heart failure	Heart transplant, steroids	25	49	3	no	Survived	Caspo	pos	6	1.1	0.60 ± 0.3	
15	2	73	F	18	Pneumonia	COPD, steroids	13	41	8	no	Death	Caspo	pos	4	2.80	1.35 ± 0.9	
16	2	63	M	24	Cardiac arrest	COPD, cirrhosis, steroids	25	50	6	no	Death	Ampho	pos	3	0.4	0.38 ± 0	
Mean ± SD		69.1 ± 8.6		17.1 ± 10.9			19.8 ± 7.9	46.3 ± 16.2	5.4 ± 3.1					3.6 ± 2.3	1.6 ± 1.8	1.1 ± 1.4	

APACHE II — Acute Physiology and Chronic Health Evaluation II; AML — Acute Myeloid Leukemia; Ampho — amphotericin; APO — anatomopathology; BAL — bronchoalveolar lavage; caspo — caspofungin; COPD — chronic obstructive pulmonary disease; Bone marrow Tx — bone marrow transplantation; F — female; GMmax — maximal galactomannan; GMmean — mean galactomannan; LOS — length of stay; M — male; neg — negative; n — number of samples; pos — positive; SAPS — Simplified Acute Physiology Score; SOFA score — Sequential Organ Failure Assessment score; Vorico — voriconazole

Table 2. Patient characteristics, differences in HOS and ICU survivors or non-survivors, and patients with or without IA

	Total (n = 160)	ICU survivors (n = 93)	ICU non-survivors (n = 67)	Sig.	HOS survivors (n = 67)	HOS non-survivors (n = 93)	Sig	no IA (n = 144)	IA (n = 16)	Sig.
Age (years)	64.5 ± 15.9	62.2 ± 17.1	67.8 ± 13.6	.027	59.8 ± 17.4	67.9 ± 13.9	.001	64 ± 16.5	69.1 ± 8.6	ns
ICU stay (days)	15.8 ± 12	16.5 ± 13	14.8 ± 10.4	ns	15.3 ± 12.9	16.1 ± 11.3	ns	15.6 ± 12.1	17.1 ± 10.9	ns
Measurements	3 ± 2.6	3.1 ± 2.8	2.8 ± 2.4	ns	2.9 ± 2.9	3 ± 2.4	ns	2.9 ± 2.6	3.6 ± 2.3	ns
GMfirst	1.1 ± 1.6	1.2 ± 1.8	1 ± 1.3	ns	1.2 ± 1.9	1 ± 1.4	ns	1.1 ± 1.7	0.8 ± 1	ns
GMmax	1.4 ± 1.8	1.4 ± 1.9	1.4 ± 1.7	ns	1.4 ± 2	1.4 ± 1.7	ns	1.4 ± 1.8	1.6 ± 1.8	ns
GMdelta	-0.1 ± 1.6	-0.4 ± 1.7	0.3 ± 1.3	.004	-0.5 ± 1.5	0.1 ± 1.5	.017	-0.2 ± 1.5	0.7 ± 1.5	.027
GMmean	0.9 ± 1.2	0.9 ± 1.2	1.1 ± 1.3	ns	0.9 ± 1.3	1 ± 1.2	ns	0.9 ± 1.2	1.1 ± 1.4	ns
CRP admission (mg dL-1)	10.4 ± 11.2	8.5 ± 9.6	13.1 ± 12.7	.010	8.6 ± 9.6	11.7 ± 12.1	.081	10.1 ± 11.4	12.6 ± 8.9	ns
BIPAP (d)	3.3 ± 3.5	3.8 ± 5.2	2.9 ± 1.9	ns	2.8 ± 2.3	3.5 ± 4	ns	2.9 ± 2.1	5.3 ± 7.6	.096
CVVH (d)	8.7 ± 6	9.2 ± 6.2	8.6 ± 6.1	ns	10.8 ± 5.9	8.3 ± 6.1	ns	9.3 ± 6.3	5.8 ± 4.2	ns
Inotropes (d)	7.6 ± 5.1	6.8 ± 4.4	8.2 ± 5.6	ns	7 ± 4.5	7.9 ± 5.4	ns	7.4 ± 4.8	8.6 ± 6.9	ns
MV (d)	12.9 ± 8.7	14.1 ± 9.5	12.1 ± 8	ns	13.5 ± 9.2	12.7 ± 8.5	ns	12.8 ± 8.3	13.9 ± 11.5	ns
PiCCO (d)	9.2 ± 5.1	9 ± 3.4	9.4 ± 5.9	ns	9.4 ± 3.6	9.2 ± 5.6	ns	9.5 ± 5	8.3 ± 5.5	ns
APACHE-II	19.3 ± 8	17 ± 7.7	22.5 ± 7.2	.000	15.4 ± 6.6	22.1 ± 7.7	.000	19.2 ± 8	19.8 ± 7.9	ns
SAPS-II	45.5 ± 16.7	40.8 ± 16	52.1 ± 15.6	.000	38.2 ± 14.9	50.9 ± 16.1	.000	45.5 ± 16.9	46.3 ± 16.2	ns
probability	38.6 ± 27.5	30.6 ± 25.1	49.6 ± 26.9	.000	25.9 ± 22.2	47.7 ± 27.4	.000	38.4 ± 27.7	40.2 ± 26.6	ns
SOFA	5.8 ± 3.5	5 ± 3	7 ± 3.8	.000	4.8 ± 3	6.6 ± 3.7	.001	5.8 ± 3.5	5.4 ± 3.1	ns
Men (%)	74 (46)	46 (49)	28 (42)	ns	36 (54)	38 (41)	.07	63 (44)	11 (69)	ns
Women (%)	86 (54)	47 (50)	39 (58)	.02	31 (46)	55 (59)	.07	81 (56)	5 (31)	ns
Culture_POS (%)	148 (93)	89 (96)	59 (88)	.067	65 (97)	83 (89)	.058	135 (94)	13 (81)	ns
MV (%)	55 (34)	49 (53)	6 (9)	.0001	40 (60)	15 (16)	.0001	45 (31)	10 (63)	ns
Neutropenia (%)	31 (19)	16 (17)	15 (22)	ns	11 (16)	20 (22)	ns	26 (18)	5 (31)	ns
Pip/tazo (%)	34 (21)	18 (19)	16 (24)	ns	11 (16)	23 (25)	ns	29 (20)	5 (31)	ns
TPN (%)	125 (78)	62 (66)	63 (94)	.0001	41 (31)	84 (90)	.0001	111 (77)	14 (88)	ns
TEN (%)	86 (54)	40 (43)	46 (69)	.001	23 (34)	63 (68)	.0001	77 (53)	9 (56)	ns
GMmax > = 0.5 (%)	98 (61)	58 (62)	40 (60)	ns	38 (57)	60 (65)	ns	89 (62)	9 (56)	ns
GMmax > = 0.8 (%)	62 (39)	35 (38)	27 (40)	ns	22 (33)	40 (43)	ns	54 (38)	8 (50)	ns
GMmax > = 1.1 (%)	53 (33)	27 (29)	26 (39)	ns	18 (27)	35 (38)	ns	45 931)	8 (50)	.07
GMmax > = 1.5 (%)	45 (28)	23 (25)	22 (33)	ns	17 (25)	28 (30)	ns	39 (27)	6 (38)	ns
IA (%)	16 (10)	5 (5)	11 (16)	.02	2 (3)	14 (15)	.009	—	—	—
HOS mortality (%)	93 (58)	26 (28)	67 (100)	.000	—	—	—	79 (55)	14 (88)	.009
ICU mortality (%)	67 (42)	—	—	—	0 (0)	67 (72)	.000	56 (39)	11 (69)	.022

ns — non significant; APACHE II — Acute Physiology and Chronic Health Evaluation II; BIPAP — bilevel positive airway pressure; CRP — C reactive protein in milligrams per decilitre by admission; CVVH — continuous veno-venous haemofiltration; d — duration in days; GM_{first} — first galactomannan sample; GM_{max} — maximal galactomannan; GM_{delta} — difference between first and maximal galactomannan; GM_{mean} — mean galactomannan; M/F — male to female ratio; n — number; MV — mechanical ventilation; PiCCO — pulse-induced contour cardiac output; SAPS — Simplified Acute Physiology Score; SOFA score — Sequential Organ Failure Assessment score; Culture pos — positive culture with Aspergillus; MV — mechanical ventilation; TPN — total parenteral nutrition; TEN — total enteral nutrition; IA — invasive aspergillosis

with proven or probable IA, only nine of the 16 (56%) had a serum GM value > 0.5 OD (range 0.5–5.2). Looking at boxplots of all GM levels (n = 474), we found significantly higher values in the patients with proven or probable IA ($P = 0.02$). The GM_{max} and GM_{delta} levels were higher in the proven group ($P = 0.07$ and $P = 0.06$ respectively), as shown in Figure 1A–C. Figure 3 shows the distribution of different mean GM levels in survivors and non-survivors according to the IA status. Only the GM_{delta} was significantly higher in patients with IA who did not survive ($P = 0.027$). We could not identify a threshold for the GM test to identify patients with or without IA. The sensitivity and specificity of the GM test at various interpretive cut-offs are presented in Table 3. The serum GM test failed to maintain an acceptable sensitivity and specificity. The best threshold was a GM >1.1 for identifying patients with IA with a specificity of 68.8% and

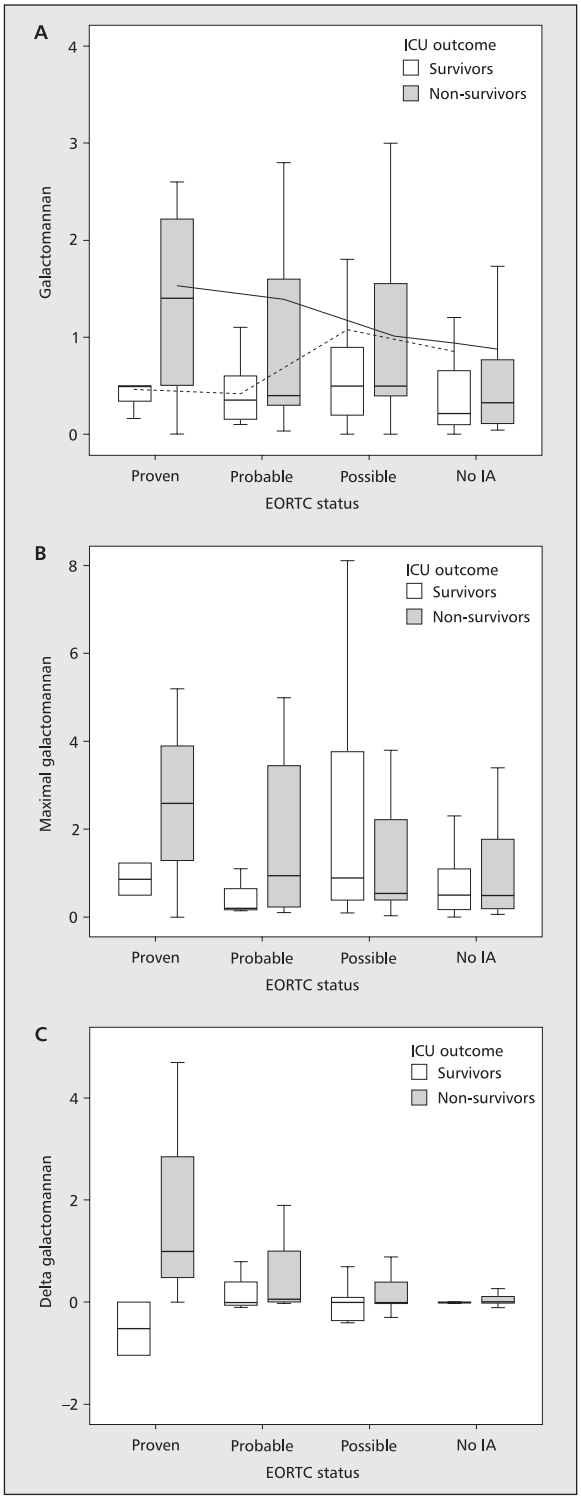

Figure 1. A — boxplot of all GM levels (n = 474) with linear regression between the mean values in ICU survivors vs non-survivors according to EORTC status. *P* = 0.02 for proven and probable between survivors and non-survivors; **B** — boxplot of maximal GM levels (n = 160) in ICU survivors vs non-survivors according to EORTC status. *P* = 0.07 for proven; **C** — boxplot of delta GM levels (n = 160) in ICU survivors vs non-survivors according to EORTC status. *P* = 0.06 for proven

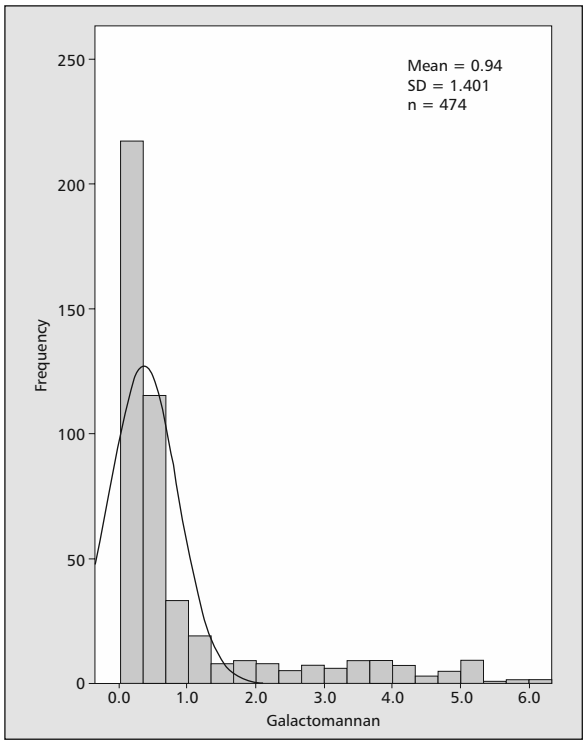

Figure 2. Histogram of all galactomannan samples

a negative predictive value (NPV) of 92.5% (*P* = 0.07). For the determination of the best predictive cut-offs, receiver operating characteristics (ROC) curves were constructed for GM in relation to the presence or absence of IA (Fig. 4). The area under the curve with a cut-off OD index of 0.5 used to diagnose IA (in neutropenic haematological patients) was only 0.573 (95% confidence interval, 0.498 to 0.648), confirming the poor performance of the GM test in the diagnosis of IA in mixed ICU patients.

PROGNOSTIC VALUE

As shown in Figure 5, the presence of IA was associated with high mortality: 14 out of 16 patients with IA died (87.5%). Eleven patients (69%) with IA died in the ICU and for patients with proven IA there was 100% hospital mortality (Fig. 6). The ICU mortality was 41.9% (n = 67) and was significantly higher than in patients without IA (*P* = 0.02). Patients who died in the ICU were mostly women (*P* = 0.02), had higher APACHE-II, SAPS-II and SOFA scores (*P* < 0.0001), and had a significant increase in GM during their stay with GM_{delta} 0.27 ± 1.26 vs –0.43 ± 1.7 in survivors (*P* = 0.004). They also received more total parenteral nutrition (TPN) compared to ICU survivors (*P* = 0.0001). The hospital mortality was 58.1% (n = 93). Patients who died in the hospital also showed a significant increase in GM during their stay, with

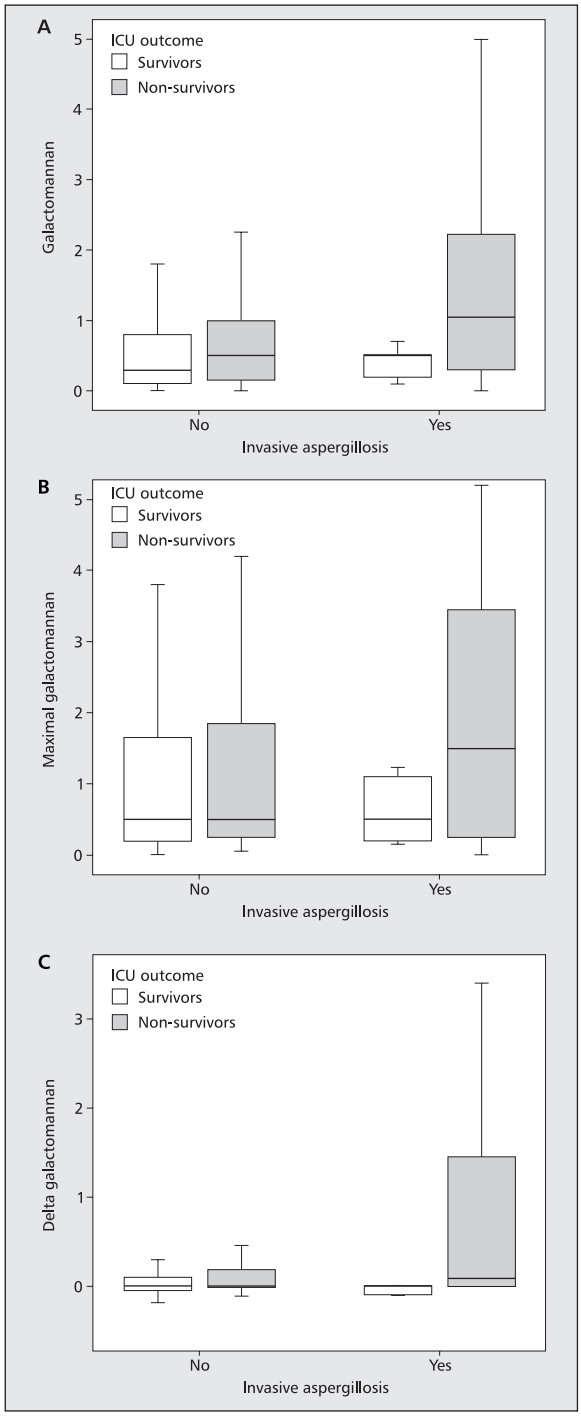

Figure 3. A — boxplot of all GM levels (n = 474) in ICU survivors vs non-survivors according to IA status. *P* = NS; **B** — boxplot of maximal GM levels (n = 160) in ICU survivors vs non-survivors according to IA status. *P* = NS; **C** — boxplot of delta GM levels (n = 160) in ICU survivors vs non-survivors according to IA status. *P* = 0.027 for IA

Table 3. Diagnostic performance for invasive aspergillosis for different galactomannan cut-off values in blood samples

GM threshold (ng mL^{-1})	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
0.5	56.3	38.2	9.2	88.7
0.8	50	62.5	12.9	91.8
1.1	**50**	**68.8**	**15.1**	**92.5**
1.5	37.5	72.9	13.3	91.3

GM — galactomannan; PPV — positive predictive value; NPV — negative predictive value

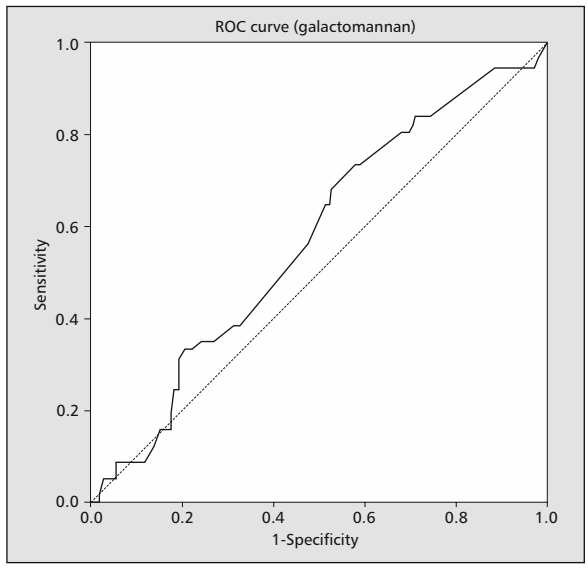

Figure 4. Receiver-operator characteristic (ROC) curves for galactomannan in blood samples for diagnosis of invasive aspergillosis. Area under the curve (AUC) for GM to diagnose IA was only 0.573 (95% confidence interval, 0.498 to 0.648)

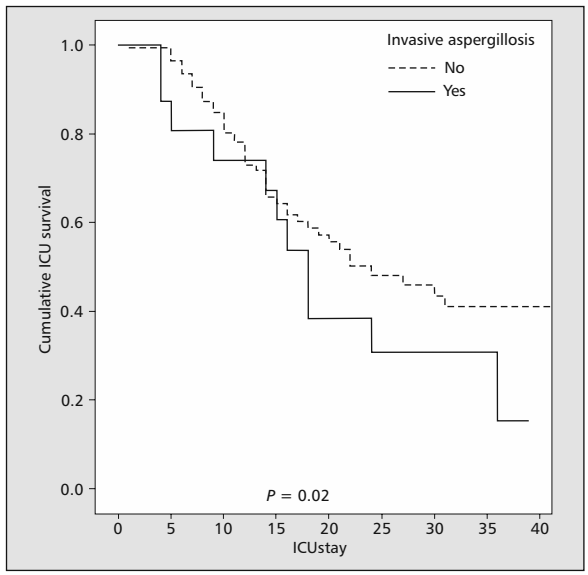

Figure 5. Kaplan Meier curve for ICU survival in patients with IA and without IA

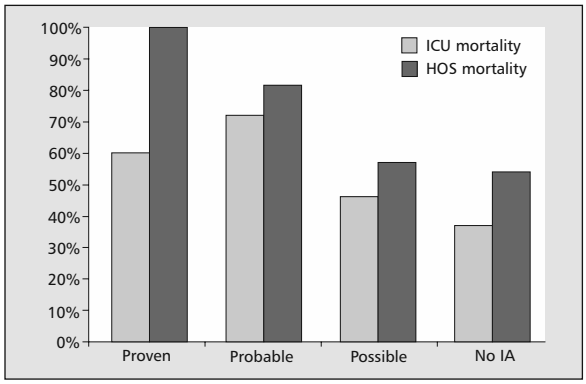

Figure 6. ICU and hospital mortality according to IA groups

GM_{delta} 0.11 ± 1.55 vs –0.48 ± 1.51 ($P = 0.017$) and had higher APACHE-II, SAPS-II and SOFA scores. The hospital mortality was also significantly higher in patients with IA ($P = 0.009$). Patients who died in the hospital received significantly more TPN ($P = 0.0001$). We observed a trend towards higher GM_{max} levels in non-survivors with IA (Fig. 3b), but this did not reach statistical significance ($P = 0.165$).

FACTORS RELATED TO FALSE POSITIVITY

When using the cut-off > 0.5 OD for IA, 89 out of 144 (62%) had a false positive GM test. There was a trend towards higher GM values in patients treated with piperacillin/tazobactam (n = 34) but this was not statistically significant. Patients on total parenteral nutrition (n = 125) had higher GM_{max} levels (1.55 ± 1.94 vs 0.88 ± 1.25, $P = 0.058$). Patients who were mechanically ventilated (MV) (n = 105) had significantly higher GM_{mean} ($P = 0.038$) and GM_{max} ($P = 0.007$) levels. Finally, neutropenic patients (n = 31) showed a significant increase in GM during their stay with GM_{delta} 0.32 ± 1.3 vs –0.43 ± 1.7 ($P = 0.07$).

DISCUSSION

In our study, we found a high incidence of proven and probable IA in mixed ICU patients (3% and 7% respectively). We confirmed the poor performance of the serum GM test in the detection and confirmation of IA in mixed ICU patients, and therefore we couldn't identify a threshold for detecting patients with IA. The best threshold was a GM > 1.1 with a specificity of 68.8% and a negative predictive value (NPV) of 92.5%. The presence of IA was associated with high mortality, of 14 out of 16 patients (87.5%), and patients who died in the ICU or in the hospital had significantly higher APACHE-II, SAPS-II and SOFA scores and a significant increase in the GM during their stay.

RISK FACTORS FOR IA

The high incidence of IA of 10% we found in critically ill patients was higher than found in previous studies where variable incidences have been reported from 0.33 to 6.9 % In a previous study, 70% of the cases did not have any underlying haematologic malignancy or neutropenia [7]. Indeed, there are several risk factors for IA in patients admitted to the ICU rather than solely the host factors mentioned in the EORTC/MSG criteria. For example, patients with prolonged treatment with corticosteroids, COPD, liver cirrhosis, solid-organ cancer, HIV infection, lung transplantation and prolonged ICU stay over 21 days [4].

An interesting finding in our study was that in the IA group there were nine patients (56%) with COPD, and all of them were treated with steroids. Two studies have analysed COPD patients with IA, and in both of the studies the outcome was very poor, reaching a mortality of 100% [11, 12]. In our study, the mortality in the COPD patients was 66%. The use of steroids for more than three weeks is considered as a risk factor in the EORTC/MSG criteria. Low dose steroids (mineralocorticoids) are often used in cases of severe and/or persistent septic shock and may improve the outcome [13], but they are known to impair the macrophage killing of Aspergillus spores and the mononuclear killing of the Aspergillus hyphae [14]. Patients with an underlying lung disease are more at risk for IA even with the use of lower doses of steroids [15].

There were also two patients with liver cirrhosis in the group of proven and probable IA. Cirrhosis has been described previously in five out of 14 patients with IA without any other risk factor [16]. Also Meersseman et al. [7] found in three patients (10%) with IA the presence of liver cirrhosis. Patients with cirrhosis have impaired phagocytosis and chemotaxis, which may play a role in the pathogenesis of IA [17].

CONFOUNDING FACTORS FOR GM

The diagnosis of IA in non-neutropenic mixed ICU patients is difficult. The use of serum GM has been successfully studied to allow a faster diagnosis of IA in neutropenic patients. However in our group of mixed ICU patients, only 19% were neutropenic. In Europe, the previous threshold for IA in neutropenic patients considered an OD ≥ 1.5 to be a positive result and an OD < 1.0 to be a negative result, while the US Food and Drug Administration interprets an OD > 0.5 uniformly as a positive result. Maertens et al. [18] showed in 2007 indeed an improvement of the test with a threshold for OD > 0.5. In our study, the five neutropenic patients in the IA group all had a GM > 0.5 OD. This was shown previously by Cordonnier et al. [19], who found that patients with neutropenia had significantly higher GM values compared to patients without neutropenia. This confirms the hypothesis that neutrophils seem to be capable of clearing galactomannan from the blood, presumably by their mannose-binding receptors [20]. But in our mixed

non-neutropenic ICU population, we found that the current GM threshold with OD > 0.5 did not allow us to discriminate between patients with and without IA. According to the ROC curve, we could not identify an optimal threshold (Fig. 4). The curve is almost diagonal, showing that the test has no capacity to discriminate between patients with or without IA. This has been shown previously in a retrospective study by Meersseman et al. [7], who found that the GM was only positive in 53% of the patients with proven IA. In 2007, the same authors found a sensitivity of only 42% for serum GM in non-neutropenic patients [8]. Together with a low sensitivity, there is a low specificity due to a high proportion of false positivity. In our population, 62% had a false positive GM test. False positivity has also been described in patients treated with beta lactam antibiotics. The combination of amoxicillin-clavulanic acid has been described as a possible antibiotic resulting in false positive GM values [21]. Also piperacillin/tazobactam can give false positive results for the GM test. We found a trend towards higher GM values in patients treated with piperacillin/tazobactam (n = 34), but this was not statistically significant. In two other studies, there was no significant association between piperacillin/tazobactam and false GM positivity. In a 2009 Brazilian study, the authors tested batches issued by five different manufacturers using the seven available tests in Brazil. Only piperacillin/tazobactam from one single manufacturer cross-reacted with GM *in vitro* [22]. Recently, a study group from Italy also could not find a significant interference with GM testing [23]. Perhaps other factors can explain false positivity of the GM test; for example, there seems to be a correlation between GM levels and total parenteral feeding, probably due to interference with the ELISA test. Patients on total parenteral nutrition (n = 125) had higher maximal GM levels (1.55 ± 1.94 vs 0.88 ± 1.25, $P = 0.058$). This could be an additional problem with using the serum GM test in the ICU, where critically ill patients may receive TPN. But on the other hand, Blijlevens et al. investigated serum GM in patients after HSCT receiving TPN; none of the samples taken was positive [24]. Another finding in our study was that patients who were mechanically ventilated had higher mean and maximal GM levels. Mechanical ventilation (MV) will affect the normal defence mechanisms of patients and can make them more vulnerable to have colonisation by Aspergillus in their lungs. There is no evidence of higher GM test in serum or BAL by mechanically ventilated patients in the literature, so these are mere speculations lacking clinical evidence or proof.

IA AND OUTCOME

The presence of IA was associated with a high mortality. Patients who died in the ICU or in the hospital had a significant increase of GM during their stay. This was also shown in other studies, where persistent elevated GM values were strongly associated with treatment failure and death [9, 25]. By using the kappa correlation coefficient test, previous studies have found a strong correlation between serum GM and outcome in patients known to have haematologic malignancies and IA. We also found higher mean GM values in non-survivors, but this was not statistically significant (Fig. 3b).

LIMITATIONS

This study has several limitations. First, albeit the total number of patients was large, the number of patients with IA was small. Second, only a few autopsies were performed to confirm the diagnosis of IA, so we might have missed some patients with a post-mortem diagnosis of IA. Third, this is a retrospective observational study in a single tertiary centre, which is a risk for inclusion and selection bias. We had an important selection bias since the samples were taken on clinical suspicion and more than ten years ago. Additionally, we have no data showing how many patients were admitted in that period, so we don't know if there was an additional selection bias made on the sample.

CONCLUSIONS

This study confirms that IA is an important problem in the ICU. We found a high incidence of proven and probable IA in a group of mixed ICU patients and the presence of IA was associated with a high mortality. The serum GM antigen detection test may not be useful in the diagnosis of IA in mixed ICU patients, according to the results of the ROC analysis. We could not define a useful threshold. There seems to be a correlation between GM levels and total parenteral nutrition, which is an additional problem for the use of the serum GM test in the ICU. Patients who died had a significant increase of GM during their stay. However, there were several limitations of this retrospective study and the results need to be validated in a prospective multicentre trial.

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