Aspergillus PCR in serum for the diagnosis, follow-up and prognosis of invasive aspergillosis in neutropenic and non-neutropenic patients

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1 ***Aspergillus* PCR in serum for the diagnosis, follow-up and prognosis of invasive**

2 **aspergillosis in neutropenic and non-neutropenic patients**

3

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15 **Running title:** *Aspergillus* PCR for the prognosis of invasive aspergillosis

16

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ABSTRACT.

We evaluated the usefulness of a serum *Aspergillus* PCR assay for the diagnosis and prognosis of invasive aspergillosis in a study involving 941 patients for a total of 5,146 serum samples. Fifty-one patients had proven/probable aspergillosis. We compared galactomannan, PCR, and mycological analysis of pulmonary samples in both neutropenic and non-neutropenic patients. PCR performed in serum yielded 66.7% sensitivity, 98.7% specificity, 75.6% positive predictive value and 98.0% negative predictive value while the galactomannan index yielded 78.4% sensitivity, 87.5% specificity, 27% positive predictive value and 98.6% negative predictive value. The inclusion of PCR in the EORTC/MSG mycological criteria permitted the reclassification of nine other cases from possible to probable aspergillosis and increased sensitivity to 71.7%. Combining the galactomannan index with serum PCR increased the detection rate of invasive aspergillosis with 88.2% sensitivity. PCR was systematically negative in 16 patients with non-invasive forms of aspergillosis (namely aspergilloma and chronic aspergillosis). Remaining PCR-positive after a period of 14 to 20 days of treatment was related to poor outcome at 30 and 90 days. Our results also indicate that, unlike the determination of the galactomannan index, the initial fungal load as determined by PCR was highly predictive of 90-day mortality, with the rate of this latter being 15.8% for patients with less than 150 copies/mL vs 73.2% for patients at or above that cut-off ($p<0.0001$). Therefore, PCR appears to be a very powerful and interesting tool for the identification of patients with invasive aspergillosis who might benefit from more intense care.

INTRODUCTION.

Invasive aspergillosis (IA) is a major threat for immunocompromised hosts. Early diagnosis and initiation of appropriate antifungal therapy are essential to improve the prognosis of the

51 disease [1, 2]. However, the diagnosis of IA remains difficult. Currently, it is frequently based
52 on a set of host, clinical and mycological criteria, such as those defined jointly by the
53 European Organisation for Research and Treatment of Cancer (EORTC) and the Mycosis
54 Study Group (MSG) [3]. In the EORTC/MSG criteria, galactomannan (GM) or β-D-glucan
55 assays are the only non-invasive mycological diagnostic methods currently available.
56 Although these assays are of interest and widely used, they lack sensitivity, especially in non-
57 neutropenic patients [4]. They also may be influenced by the use of certain medical devices
58 [5] or antibiotics [6, 7], leading to a large number of false positive results. GM detection has
59 also been reported in invasive fungal diseases not due to *Aspergillus* [8-10]. Moreover, β-D-
60 glucan is present in many fungal species and is therefore not specific to aspergillosis. Other
61 tools have been developed to improve diagnosis, including in particular real-time PCR in
62 blood samples to detect circulating *Aspergillus* DNA. Several studies have shown the
63 potential interest of this PCR approach, but due to a lack of standardisation, it has not yet
64 been included in the EORTC/MSG mycological criteria. Very recently however, a panel of
65 experts argued for its inclusion in those criteria [11].
66 For the present study, we aimed at evaluating the performance of an in-house *A. fumigatus*
67 real-time PCR assay using 1 mL volume of serum for the diagnosis of IA in at-risk patients,
68 both neutropenic and non-neutropenic, and comparing the PCR results with those of the GM
69 assay to determine their 30 and 90-day prognostic contributions.
70
71 **MATERIALS AND METHODS.**
72 **Design.** A retrospective single-centre analysis was performed between February 2012 and
73 October 2014 in La Pitié-Salpêtrière hospital, in Paris, France.
74 **Patients.** All patients who are at risk of IA are routinely subjected to monitoring with a
75 serologic assay for the detection of GM antigen and serological PCR for the detection of *A.*

76 *fumigatus* DNA. For the present study, we focused on patients with proven/probable IA
77 according to EORTC/MSG criteria [3], extended to include the additional criteria of alcoholic
78 liver cirrhosis, a long stay in the ICU and severe acute respiratory distress syndrome as host
79 factors, as already reported [12, 13]. GM either in serum or in broncho-alveolar lavage (BAL)
80 was used as mycological criterion for probable case. GM determination and PCR results were
81 also available for some patients experiencing non-invasive aspergillosis.
82 **PCR.** We used a real time PCR that targets a previously described 67-bp segment of a 28S
83 ribosomal RNA coding DNA [14, 15], for which the primer sequences were
84 5'CTCGCAATGTATCACCTCTCGG3' and 5'TCCTCGGTCCAGGCAGG3' and the probe
85 was 5'(6FAM)TGTCTTATAGCCGAGGGTGCAATGGG(TAMRA)3'. DNA extraction was
86 performed on 1 mL of serum with the MagNA Pure Compact large volume kit on a MagNA
87 Pure device (Roche). Elution volume was 50 µL. Amplification was performed on the 7500
88 Fast Real-Time PCR System (Applied Biosystems). Quantification was achieved using 5
89 serial ten-fold dilutions of the plasmid PGEMT Easy-Afu28S containing the target. The final
90 PCR result was expressed in numbers of copies per mL of serum. We used an internal control
91 in our assay for all wells (TaqMan exogenous Internal Positive Control) as well as an
92 extraction control (albumin gene) for each sample. All PCRs were performed in duplicate. A
93 single positive well was considered as a positive result.
94 **Galactomannan determination.** The GM index was determined by enzyme immuno-assay
95 (BioRad) according to the manufacturer's recommendations. A result was considered positive
96 after two determinations, performed on two different assays but on the same sample, showing
97 both an index above 0.5 for serum and 1 for BAL.
98 **Statistical analysis.** Tests were performed using GraphPad Prism 5 and the free online site
99 BiostaTGV (http://marne.u707.jussieu.fr/biostatgv/).
100

RESULTS.

Patients.

Over the study period, GM assay and *A. fumigatus* PCR were performed in 970 patients (Figure 1). Clinical data were available for 941 patients (5,146 serum samples). A diagnosis of proven or probable IA was made respectively for 6 and 45 patients according to the extended EORTC/MSG criteria. Moreover, a non-invasive form of aspergillosis was diagnosed in 16 patients. Also, although the study was not focused on an exhaustive identification of all possible cases, we did register diagnoses of IA based on host factors and clinical evaluation for nine others patients, classifying them as possible IA according to the EORTC/MSG criteria. These patients had positive serum PCR.

Characteristics and outcomes of patients with aspergillosis (Table 1).

Among the 51 patients with proven/probable aspergillosis, 19 were female and 32 male. Their median age was 56 years (range 20-82). Twenty-two patients (43%) were neutropenic (absolute neutrophil count <500/µL) at the time of diagnosis. Underlying conditions were haematopoietic stem cell transplant (n=17; 33.3%), haematological malignancies (n=13; 25.5%), heart transplantation (n=9; 17.7%), liver transplantation (n=3; 5.9%), kidney transplantation (n=1; 2%) and liver/kidney transplantation (n=1; 2%). Others risk factors were present in seven patients as follows: severe acute respiratory distress syndrome (n=2; 3.8%), oncological diseases (n=2; 3.8%), alcoholic liver cirrhosis (n=1; 2%), cardiogenic shock (n=1; 2%), multi-organ failure and long stay in intensive care unit (n=1; 2%). Overall three-month mortality was 49%. Neutropenic patients had a rate of mortality of 54.5% (12/22) vs 44.8% (13/29) for non-neutropenic patients (p=0.49 by Fischer exact test).

125 **Performance of the galactomannan index, mycological examination of respiratory**
126 **sample and PCR for the diagnosis of proven/probable aspergillosis.**

127 A diagnosis of proven/probable IA (according to the extended EORTC/MSG criteria; n=51)
128 was made for 40 patients by a positive GM index (Figure 2). PCR was positive in 34 patients
129 (including 11 with a positive GM in serum, 4 with a positive mycological analysis of
130 respiratory samples, 18 with both positive GM in serum and positive mycological analysis of
131 respiratory samples and 1 with an isolated positive GM in BAL), including five with a
132 negative serum GM index. In one patient with a positive PCR, the diagnosis of probable
133 aspergillosis was based only on a positive GM index in a bronchoalveolar lavage sample.
134 Sensitivities of the serum GM index, mycological examination and serum PCR were 78.4%
135 (95% confidence interval [CI], 67.1-89.7%), 62.7% (95% CI, 49.4-76%), and 66.7% (95% CI,
136 53.8-79.6%) respectively. Of note, sensitivity of GM and PCR in case of proven aspergillosis
137 was similar (83.3%; 5/6 patients). Among the patients for whom the diagnosis of any clinical
138 form of aspergillosis was excluded by clinicians, there were 108 with positive GM indices, 11
139 with positive PCR results, and 2 with positive GM indices and positive PCR results.
140 Specificities of GM and PCR were 87.5% (95% CI, 85.3-89.7%) and 98.7% (95% CI, 97.9-
141 99.5%), respectively. PCR yielded 75.6% positive predictive value (PPV) (95% CI, 63.1-
142 88.1%) and 98.0% negative predictive value (NPV) (95% CI, 97.1-98.9%) while the
143 galactomannan index yielded 27% PPV (95% CI, 19.8-34.2%) and 98.6% NPV (95% CI,
144 97.8-99.4%). No differences were observed between PCR and GM as concerns the precocity
145 of diagnosis (data not shown).

146

147 **PCR results for non-invasive forms of aspergillosis.**

148 Among the 16 non-invasive aspergillosis patients, 10 were immunocompetent, 3 had
149 metastatic malignancy, 2 had solid organ transplantation and one had alcoholic liver cirrhosis.

150 Clinical forms were simple aspergilloma (n=7), colonisation (n=4), chronic cavitary
151 aspergillosis for (n=4) and chronic bronchitis (n=1). In addition to clinical condition, host
152 history and radiological features, the patient diagnoses were further supported by mycological
153 examination (n=11), the presence of anti-*Aspergillus* antibodies (n=1) or both (n=4). None of
154 these patients had positive serum GM or PCR.
155
156 **Addition of PCR to the mycological criteria.**
157 Of the 9 patients treated for IA but classified as possible cases due to a lack of EORTC/MSG
158 mycological criteria, 7 were male and 2 were female. Six patients were neutropenic (five
159 haematological malignancies and one haematopoietic stem cell transplant) and 3 were not
160 (one liver transplantation, one kidney transplantation and one severe acute respiratory distress
161 syndrome). All 9 of these patients had positive PCR results.
162 Thus, considering PCR as a mycological criterion would have enabled the reclassification of
163 9 patients from possible to probable IA, increasing the number of patients with
164 proven/probable IA to 60 (Table 1 and 2). In this scenario, GM determination and PCR would
165 have been positive in 40 and 43 of the 60 patients respectively, conferring sensitivities of
166 66.7% (95% CI, 54.8-78.6%) and 71.7% (95% CI, 60.3-83.1%) and specificities of 87.7%
167 (95% CI, 85.5-89.9%) and 98.8% (95% CI, 98.1-99.5%) for GM and PCR respectively. PCR
168 would yield 79.6% PPV (95% CI, 68.9-90.3%) and 98.0% NPV (95% CI, 97.1-98.9%) while
169 the galactomannan index would yield 27% PPV (95% CI, 19.8-34.2%) and 97.5% NPV (95%
170 CI, 96.4-98.6%).
171
172 **Neutropenic versus non-neutropenic.**
173 Considering these 60 patients, PCR sensitivity tended to be better in neutropenic patients
174 (82.1%) than in non-neutropenic patients (62.5%). The difference did not reach statistical

175 significance (p=0.09) but the power of the test (P=39%) was insufficient to conclude. PCR
176 specificity did not differ between the two groups. As for the GM index, sensitivity did not
177 differ between the two groups but specificity was significantly lower in neutropenic patients
178 (83.9%) compared to non-neutropenic patients (91.2%) (p<0.005).
179
180 **Effect of antifungal therapy.**
181 Among our 60 patients with IA, 49 have at least one sample before the targeted antifungal
182 therapy initiation. All patients had a sample after treatment initiation except for 5 patients
183 who died early. At the time of diagnosis, 24 patients received antifungal therapy: 12 had
184 empirical therapy with caspofungin and 12 have more targeted anti-*Aspergillus* therapy with
185 voriconazole or amphotericin B. Among the patients who received antifungal drugs, PCR and
186 GM yield similar sensitivity of 70.8% (17/24) and there was also no difference according to
187 the antifungal drug that was used. Among the 36 patients without antifungal therapy,
188 sensitivity was 72.2% (26/36) for PCR and 63.9% for GM (23/36). The difference is not
189 statistically significant (p=0.6). When comparing patients with antifungal therapy and patients
190 without, there were also no statistically significant difference of sensitivity either for PCR, or
191 for GM.
192
193 **Variation of the fungal load after treatment initiation and prediction of outcome.**
194 Real time PCR allows the quantification of fungal load with results expressed as the number
195 of *Aspergillus* gene copies per mL of serum. GM results are presented as an index which can
196 be interpreted quantitatively. Considering this, we assessed variations (decreases or increases)
197 in PCR and the GM index to determine their ability to predict treatment efficacy and 30- and
198 90-day mortality.

199 Concerning the response to targeted antifungal therapy, we observed no early variations in
200 PCR results as fungal loads did not decrease quickly after the initiation of treatment, even
201 when outcomes were favourable. Moreover, an increase or decrease in the number of copies
202 in the first week was not related with day 30 or day 90 outcome. However, patients who
203 become PCR negative (and one who had cleared more than 99% of the initial load) between
204 day 14 and day 20 after treatment initiation were all alive at day 30 (n=9) while those who
205 remained PCR positive during this period had poor outcomes, with 80% (4/5) mortality at day
206 30 (Table 3). This result was highly significant (p<0.005 by Fischer exact test). PCR results
207 between day 14 and day 20 were also predictive of day 90 outcome (Table 3).
208

209 **Initial fungal load and prediction of outcome.**

210 We also assessed initial fungal loads as predictors of outcome. ROC curves for the evaluation
211 of fungal load as a marker of 90-day mortality in invasive aspergillosis indicated that the 150
212 copies/mL cut-off offered the most efficient value (Figure 3a). Patients with PCR results
213 strictly below 150 copies/mL had a higher probability of survival 90 days after the diagnosis
214 (n=30/41; 73.2% survival), compared to those with PCR results equal to or above this cut-off
215 (n=3/19; 15.8% survival; median survival 20 days) (Figure 3b). This result was highly
216 statistically significant (p<0.0001 with Log-rank test). The test had a hazard ratio of 0.14
217 (95% CI of ratio: 0.05 to 0.34). Similar results were obtained when all patients were
218 considered i.e. including the 17 patients with negative PCR in the '<150 copies/mL' group.
219 There were no differences between these two groups in terms of age, sex ratio, underlying
220 diseases or antifungal therapy (Table 1). There were also no differences concerning the
221 interval between sampling and the start of targeted antifungal therapy. In comparison, among
222 patients with initial positive GM, patients with GMs below 2 (the most efficient value) appear

223 to have more favourable outcomes than others but the difference was not statistically
224 significant (p=0.19 by Log-rank test) (Figure 3c).
225
226 **DISCUSSION.**
227 PCR was originally excluded from the 2008 EORTC/MSG definitions of invasive fungal
228 diseases, but expert consensus now considers that this method is 'mature' enough for its
229 inclusion [11]. Meta-analyses have indeed demonstrated that PCR offers sensitivity ranging
230 from 77% to 88% and specificity from 75% to 94.1%, rates similar to those attained with GM
231 assays [16-18]. Moreover, PCR permits the use of quality control and inter-laboratory checks,
232 and provides a more robust quantification assessment than does the determination of the GM
233 index.
234 The results of the present study add to this evidence, with PCR sensitivity reaching 71.7% and
235 specificity 98.7%. In our study, PCR appeared to offer an interesting PPV, i.e., 79.6% for all
236 patients, in stark contrast to the GM index, where a high false positive rate, particularly in
237 neutropenic patients, led to a poor overall PPV of 27%. PCR and GM both offered interesting
238 negative predictive values above 97%. PCR was more sensitive in neutropenic patients
239 (82.1%) than it was in non-neutropenic patients (62.5%) but the difference was not significant
240 (p=0.09). Increasing the number of patients could lead to a significant result. Nevertheless,
241 our study shows that PCR is also useful in non-neutropenic patients and non-haematological
242 populations such as solid organ transplant recipients. As previously reported, our result
243 indicate that performing both GM determination and PCR on the same sample increases the
244 sensitivity [18].
245 In 2010, Koo et al reported a relation between GM and outcome [19] and in 2012 Bergeron et
246 al reported that patients with poor outcomes 45 days after the initiation of antifungal treatment
247 had high baseline serum GM [20]. We however found only a non-statistically-significant

248 trend for the GM index in the determination of outcomes. Nevertheless, Bergeron et al also
249 found no association between outcome and *Aspergillus* DNA detection while our work
250 supports that the initial level of *Aspergillus* DNA is highly predictive of the 90-day mortality
251 rate. As concerns our results more specifically, we found that a PCR threshold of 150
252 copies/mL could discriminate patients with low (below the threshold) or high (above)
253 probabilities of 90-day mortality. PCR thus has a much greater discriminative capacity than
254 does the determination of the GM index. This result could be useful for identifying patients
255 who may benefit from more intensive care and designing further clinical study. We also
256 showed that PCR quantification is useful in follow-up to predict outcomes. After only one
257 week of therapy, an increase or decrease in the number of copies is not relevant for the
258 assessment of treatment efficacy or outcome. However, PCR results after 2 weeks of
259 treatment did appear to have relevance. In our study, patients who had a negative PCR (to
260 which we add one patient who had cleared more than 99% of the initial load) between days 14
261 and 20 were all alive at day 30 and 7 of them were still alive at day 90 vs none of the five
262 patients who remained PCR positive.
263 In a work initiated by the European Aspergillus PCR Initiative, White et al clearly
264 demonstrated that PCR performed on plasma gives better results than PCR performed on
265 serum [21]. In consideration of this evidence, we are now changing our methods. The PCR
266 that we used in the study was specific to the *fumigatus* species and thus it would not have
267 amplified the genomes of others species such as *flavus*, *niger* or *nidulans*. This may explain at
268 least in part the relative lack of sensitivity for PCR in our study, as non-*fumigatus* species
269 may account for more than 25% of disease [22]. We did however retrieve the causative
270 species in 32 of our cases and, strikingly, all were *fumigatus*. Going forward, the utility of
271 PCR in IA and the determination of cut-offs will have to be evaluated in other laboratory
272 specimens, such as cerebrospinal fluid and bronchoalveolar lavage.

273 **CONCLUSION.**

274 *Aspergillus* DNA detection in serum by PCR is a interesting tool for the diagnosis of invasive
275 aspergillosis in both neutropenic and non-neutropenic patients and should be used
276 concomitantly with the galactomannan index in at-risk populations. Moreover, PCR allows
277 quantification of fungal load and our results indicate that a threshold of 150 copies/mL is very
278 powerful to discriminate patients with low (below the threshold) or high (above) probabilities
279 of 90-day mortality. So, initial fungal load and variations in fungal load during treatment as
280 determined by PCR are robust predictive markers of mortality that may be used to identify
281 patients who might benefit from closer and more attentive care.

282

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285 Mycology (SFMM: Société Française de Mycologie Médicale) held 21 May 2015. The
286 presentation was awarded the Oral Communication Prize.

287 A presentation of part of the data has been performed as an oral communication at the Trends
288 in Medical Mycology international congress, held in October 2015 in Lisbon, Portugal.

289

290 **TRANSPARENCY DECLARATION.** The authors declare no conflicts of interest.

291

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293

294 **FIGURES CAPTION**

295 **Figure 1: flow chart describing the number of patients and samples included in the**
296 **study.**

297 [1]: extended EORTC/MSG criteria included host factors as published in 2008 plus several
298 other host factors now recognised as leading to a risk of developing invasive aspergillosis,
299 namely alcoholic liver cirrhosis, severe acute respiratory syndrome, long stay in intensive care
300 unit, and solid organ cancer

301 [2]: the study design did not include an exhaustive collection of possible cases; we present only
302 the possible cases with positive PCR results that were considered as invasive aspergillosis by
303 clinicians and treated as such.

304

305 **Figure 2: Venn diagram showing data for patients with invasive aspergillosis (n=60).**
306 Diagram shows data for patients for whom PCR products and galactomannan in sera as well
307 as mycological analysis of respiratory samples were available. Among the 10 patients with
308 only positive PCR, one was positive for galactomannan in bronchoalveolar lavage samples.

309

310 **Figure 3: serum *Aspergillus* PCR is highly predictive of 90-day mortality in invasive**
311 **aspergillosis**.
312 3a. ROC curve for the evaluation of PCR (square) or GM (triangle) as a marker of 90-day
313 mortality in invasive aspergillosis. The 150 copies/mL cut-off offers the most efficient value
314 and an area under the curve of 0.837. For GM index cut-off of 2 (the most efficient value) is
315 related to a small area under the curve of 0.546.
316 3b. Patients with initial fungal loads below 150 copies/mL (n=41; 73.2% survival) have more
317 favourable outcomes than other patients (n=19; 15.8% survival): $p<0.0001$ by Log-rank
318 (Mantel-Cox) test; hazard ratio 0.14 (95% CI of ratio 0.05 to 0.34). 3c. Patients with initial
319 GMs below 2 (n=28; 50% survival) appear to have more favourable outcomes than others

320 (n=12; 25% survival) but the difference is not statistically significant: p=0.19 by Log-rank
321 (Mantel-Cox) test; hazard ratio 0.5 (95% CI of ratio 0.20 to 1.29).
322
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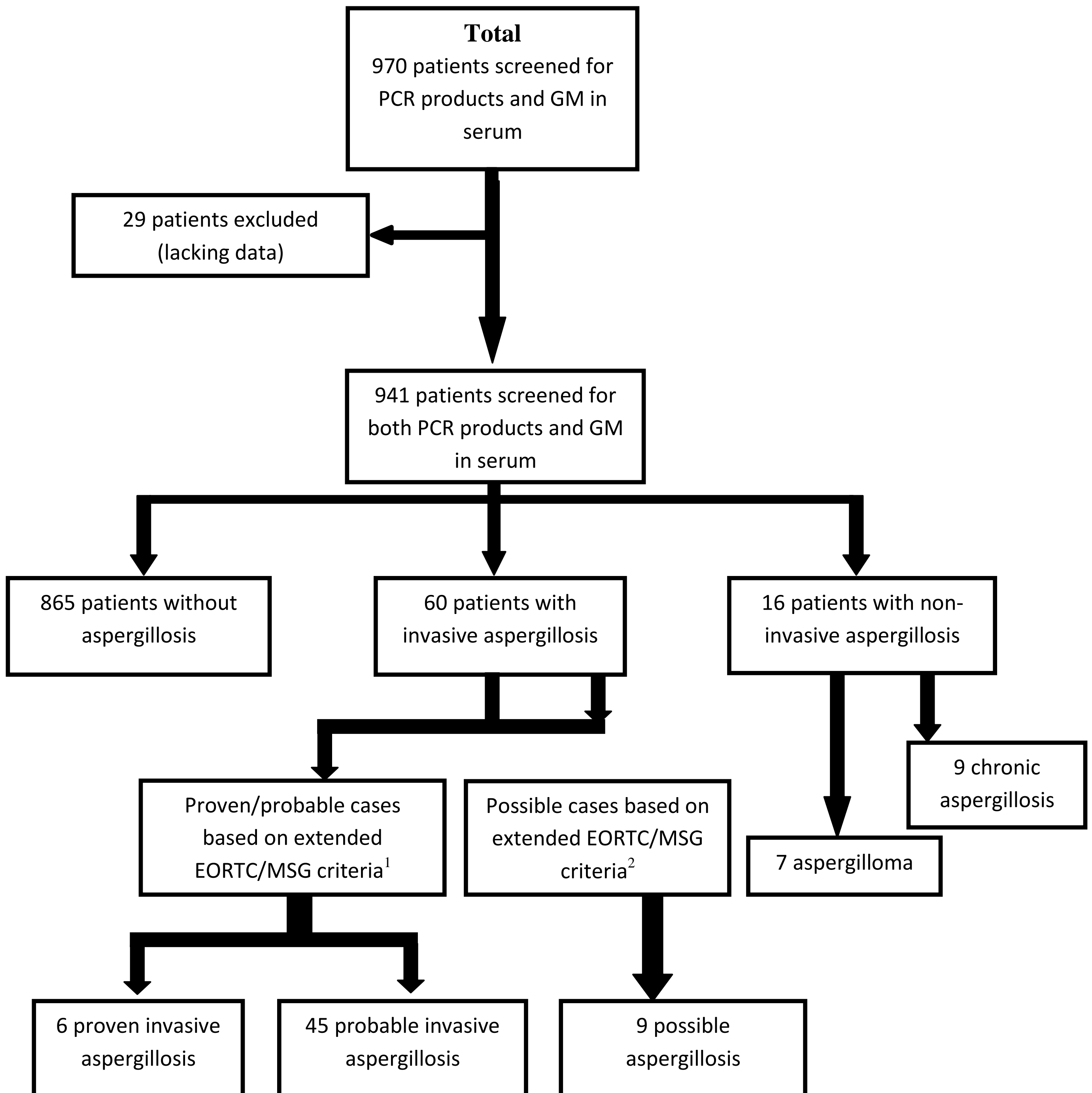
		Characteristics of patients with proven/probable invasive aspergillosis				
		EORTC/MSG criteria		According to the *Aspergillus* PCR level		
		without PCR	with PCR	Number of copies		
				<150	≥150	p
Patients; n		51	60	41	19	
Haematological malignancies (%)		13 (25.5)	18 (30)	10 (24,4)	5 (26,3)	0.87
Haematopoietic stem cell transplant (%)		17 (33.3)	18 (30)	10 (24,4)	6 (31,6)	0.78
Antifungal therapy; n (%)[a]	Azole based	36 (70.5)	42 (70)	31 (75,6)	11 (57,9)	0.6
	Non-azole based	13 (25.5)	16 (26.7)	10 (24,4)	6 (31,6)	
SOT recipient (%)		14 (27.4)	16 (26.7)	9 (22)	5 (26,3)	0.96
Others (%)		7 (13.7)	8 (13.3)	5 (12.2)	3 (15.8)	0.7
Interval between time of sample and start of targeted antifungal therapy in days; mean		0.65	0.67	0.8	0.37	0.7
3-months mortality (%)		25 (49)	27 (45)	11 (26.8)	16 (84.2)	<0.0001

Table 1: characteristics of patients with proven/probable invasive aspergillosis according to EORTC/MSG criteria and the *Aspergillus* PCR level

[a] : two patients in the ">150 copies/mL" group died before the start of antifungal therapy

Figure 1



Method	Neutrophil status	Group	Number of samples with result		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	p value [b]	
			Positive	Negative						
Galactomannan	All patients	IA[a]	40	20	66.7	87.7	27	97.5		
		Non IA	108	773						
	Neutropenic	IA	19	9	67.8	83.9	22	97.5	0.93	in comparison with non-neutropenic
		Non IA	67	350					<0.005	in comparison with non-neutropenic
	Non-neutropenic	IA	21	11	65.6	91.2	33.9	97.5		
		Non IA	41	423						
Mycological examination	All patients	IA	32	14	69.6	NA[c]	NA[c]	NA[c]		
	Neutropenic		8	9	47					
	Non-neutropenic		24	5	82.7					
PCR	All patients	IA	43	17	71.7	98.8	79.6	98		
		Non IA	11	870						
	Neutropenic	IA	23	5	82.1	98.1	74.2	98.8	0.09	in comparison with non-neutropenic
		Non IA	8	409					0.09	in comparison with non-neutropenic
	Non-neutropenic	IA	20	12	62.5	99.4	87	97.5		
		Non IA	3	461						

Table 2: Performance of PCR to detect *Aspergillus fumigatus* in serum, determination of galactomannan index in serum and mycological examination of respiratory samples for the diagnosis of invasive aspergillosis in 60 patients treated for proven/probable invasive aspergillosis according to extended EORTC/MSG criteria with the addition of PCR in the mycological criteria

[a] IA: invasive aspergillosis; criteria used for classification were those defined jointly by the EORTC/MSG consensus and published in 2008 with, additionally, the inclusion of PCR as a mycological criteria and minor modifications for host factors (e.g. inclusion of alcoholic liver cirrhosis)

[b] as calculated by the Chi-square test between neutropenic and non-neutropenic patients

[c] not available

Figure 2

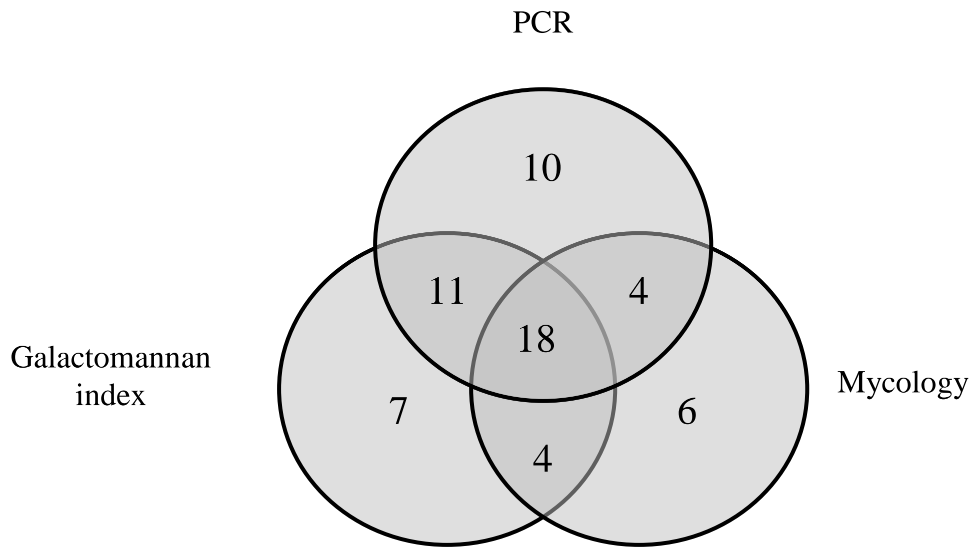
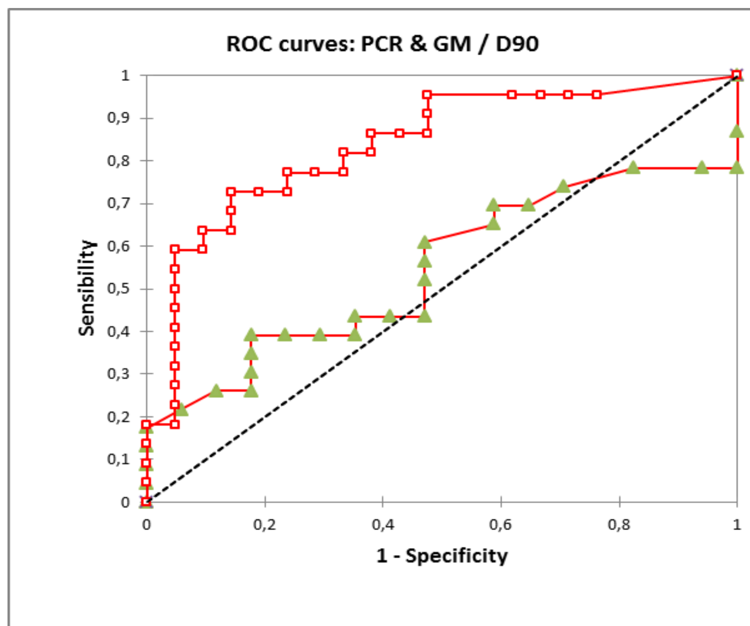
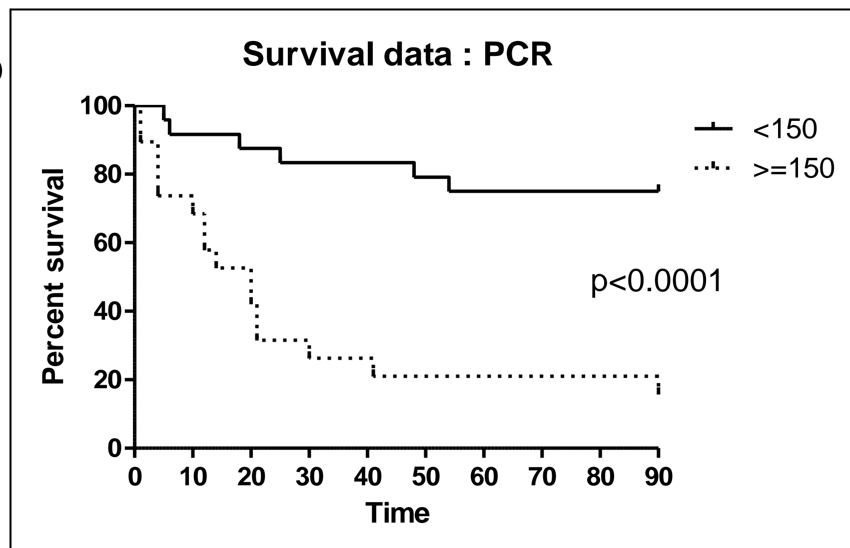


Figure 3: **serum *Aspergillus* PCR is highly predictive of 90-day mortality in invasive aspergillosis**.

3a



3b



3c