Invasive Aspergillosis: Performance of New and Established Diagnostic Approaches with Same-Day Blood and Bronchoalveolar Lavage -- a Prospective Cohort Study

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Objectives: *Aspergillus* spp. and other molds have been shown to induce elevated levels of several cytokines. It remains unknown whether these cytokines hold value for clinical routine and enhance diagnostic performances of established and novel biomarkers/molecular tests for IA and other IMI.

Aims: To determine the diagnostic potential of a number of cytokines, as well as established and emerging tests for IA and IMI in patients with underlying hematological malignancies in a setting that uses mold-active prophylaxis.

Methods: This cohort study included 106 prospectively enrolled (2014-2017) adult cases with underlying hematological malignancies and suspected pulmonary infection undergoing bronchoscopy. Serum samples were collected within 24 hours of BALF sampling. Both, serum and BALF samples were used to evaluate diagnostic performances of the *Aspergillus*-specific LFD, *Aspergillus* PCR, GM, BDG, and a bundle of cytokines for IA and IMI, classified according to the revised EORTC/MSG criteria.

Results: Among the 106 cases, 11 had probable IA, 32 possible IA (of which 7 had probable IMI), and 63 no evidence for IA. Combinations of serum IL-8 with either BALF LFD (sensitivity 100%, specificity 94%) or BALF PCR (sensitivity 91%, specificity 97%) were highly sensitive and specific for differentiating probable IA from no IA. Serum IL-8 showed also potential for differentiating patients with possible/probable IMI from those without IMI (AUC 0.641, $p < 0.014$), while serum and BALF GM (AUC 0.607, $p > 0.05$ and AUC 0.503, $p > 0.05$, respectively) and serum BDG (AUC 0.514, $p > 0.05$) did not.

Conclusion: Our study indicates that serum IL-8 testing may be a valuable addition to clinical routine for diagnosing IA in high risk patients who receive mold-active antifungals.

Figure 1: ROC curve analysis for IL-6 & IL-8 in serum & BALF samples for cases with probable IA vs. no evidence for IA.

Table 1 (above): Performance of diagnostic tests in same-day serum and BALF samples, as well as combinations, for differentiating probable IA (n=11) vs. cases with no evidence for IA (n=63), ordered by DOR.

Table 2 (below): Characteristics of cases with probable IA and no evidence for IA. #: Included in cases without evidence for IA 4 CLL, 2 primary myelofibrosis, 2 Hodgkin's lymphoma and 1 anaplastic anemia. §: probable IA = 1 bacterial; no IA = 8 bacterial, 3 *Pneumocystis*, 1 *Toxoplasma*.

Abbreviations: AF=antifungal; ALL=acute lymphocytic leukemia; AML=acute myelogenous leukemia; Asp.=*Aspergillus*; AUC=area under the curve; BALF=bronchoalveolar lavage fluid; BDG= β -D-glucan; DOR=diagnostic odds ratio; GM=galactomannan; GvHD=graft versus host disease; IA=invasive aspergillosis; IL=interleukin; IMI=invasive mold infection; LFD=*Aspergillus* specific lateral-flow-device test; MDS=myelodysplastic syndrome; MM=multiple myeloma; NHL=Non-Hodgkin lymphoma; NPV=negative predictive value; PPV=positive predictive value; ROC=receiver operating characteristics; SCT=stem cell transplantation.

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Test / test combination	Sensitivity	Specificity	PPV	NPV	DOR
Serum IL-8 (>300 pg/mL)	45% (5/11)	98% (62/63)	83% (5/6)	91% (62/68)	51.7 (5.1-518)
Serum IL-8 (>60 pg/mL)	55% (6/11)	92% (58/63)	55% (6/11)	92% (58/63)	13.9 (3.1-62.2)
Serum IL-8 (>14 pg/mL)	82% (9/11)	63% (40/63)	28% (9/32)	95% (40/42)	7.8 (1.6-39.4)
Serum IL-6 (>40 pg/mL)	73% (8/11)	70% (44/63)	30% (8/27)	94% (44/47)	6.2 (1.5-25.9)
Serum LFD (45 min)	9% (1/11)	97% (61/63)	33% (1/3)	86% (61/71)	3.0 (0.3-36.9)
Serum BDG (>80 pg/mL)	45% (5/11)	75% (47/63)	24% (5/21)	89% (47/53)	2.4 (0.7-9.1)
Blood <i>Aspergillus</i> PCR	0% (0/10)	100% (55/55)	-	85% (55/65)	-
Serum LFD (15 min)	0% (0/10)	98% (54/55)	-	84% (54/64)	-
BALF LFD (10 min)	73% (8/11)	95% (60/63)	73% (8/11)	95% (60/63)	53.3 (9.2-310)
BALF <i>Aspergillus</i> PCR	27% (3/11)	98% (58/59)	75% (3/4)	91% (58/64)	21.8 (2.0-235)
BALF LFD (15 min)	73% (8/11)	87% (55/63)	50% (8/16)	95% (55/58)	18.3 (4.0-83.8)
BALF IL-8 (>556 pg/mL)	91% (10/11)	48% (30/63)	23% (10/43)	97% (30/31)	9.1 (1.1-75.3)
BALF IL-8 (>1000 pg/mL)	73% (8/11)	67% (42/63)	28% (8/29)	93% (42/45)	5.3 (1.3-22.2)
Serum IL-8 (>300 pg/mL) +/- BALF LFD (10 min)	100% (11/11)	94% (59/63)	73% (11/15)	100% (59/59)	304 (15.3-6042)
Serum IL-8 (>300 pg/mL) +/- BALF LFD (15 min)	100% (11/11)	86% (54/63)	55% (11/20)	100% (54/54)	132 (7.2-2432)
Serum IL-8 (>300 pg/mL) +/- BALF Asp. PCR	91% (10/11)	97% (57/59)	83% (10/12)	98% (57/58)	285 (23.6-3447)

	Probable IA (n=11)	No IA (n=63)
Female / Male	6 (55%) / 5 (45%)	34 (54%) / 29 (46%)
Age: Median (Range)	55 (49-74)	57 (27-85)
AML or MDS	6 (50%)	23 (37%)
NHL	2 (18%)	18 (29%)
MM	1 (9%)	8 (13%)
ALL	2 (18%)	5 (8%)
Others [#]	0	9 (14%)
SCT: Autologous / Allogeneic	2 (18%) / 3 (27%)	7 (11%) / 13 (21%)
GvHD	2 (18%)	7 (11%)
Systemic corticosteroid treatment within 14 days of sampling	6 (55%)	18 (29%)
Positive diagnostic test for viral infections within 14 days of sampling	5 (45%)	18 (29%)
Positive diagnostic test for relevant bacterial pathogens/ <i>Pneumocystis</i> or <i>Toxoplasma</i> in BALF [§]	1 (9%)	12 (19%)
Neutropenia (<500/ μ L) in days: ≤ 10 / > 10	3 (27%) / 5 (45%)	11 (17%) / 8 (13%)
Mold active AF prophylaxis/ treatment (within 24h before sampling and ≥ 2 days)	10 (91%)	45 (71%)

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