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SUPPLEMENT ARTICLE

Defining Galactomannan Positivity in the Updated EORTC/MSGERC Consensus Definitions of Invasive Fungal Diseases

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The consensus definitions of invasive fungal diseases from the EORTC/MSGERC were recently revised and updated. They now include consensus cutoff values for the galactomannan test that support the diagnosis of probable invasive aspergillosis. In this supplement article, we provide a rationale for these proposed thresholds based on the test's characteristics and performance in different patient populations and in different specimen types.

Keywords. invasive aspergillosis; galactomannan; consensus definitions; thresholds.

The EORTC/MSGERC consensus definitions of invasive fungal diseases were first published in 2002 [1] and have since been widely adopted in clinical research, including epidemiologic studies, validation of diagnostic tests, and trials on antifungal drugs. In addition, regulatory agencies such as the Food and Drug Administration (FDA) and the European Medicines Agency have accepted these diagnostic criteria (in particular, for proven and probable diseases) for defining the target population of clinical trials that evaluate novel antifungal agents. Of course, continuing advances in the diagnostic technology and the identification of new populations at risk have led to revisions of this document. By the end of 2019, the second revision of these consensus definitions, including new host factors, radiologic features, and microbiologic tests, was published [2]. As clearly stated in the original 2002 manuscript and re-emphasized in the 2008 and 2019 revision documents [1–3], these definitions are not intended to direct or guide patient care but should be used exclusively to increase the likelihood of having the fungal disease of interest in patients included into epidemiologic, diagnostic, or therapeutic research [4, 5].

Aspergillus antigen detection was a mycologic criterion to classify probable invasive aspergillosis (IA) cases in the 2002 consensus definition, although, at that time, a commercial assay was not widely available [1]. In the 2008 revised definitions, detection of *Aspergillus* galactomannan in plasma, serum, bronchoalveolar lavage fluid (BALF), or cerebrospinal fluid (CSF) was considered mycologic evidence that supported a probable diagnosis, but a cutoff value was not provided [3]. As a consensus could not be reached on the galactomannan cutoff, the optical density index (ODI) value that was recommended by the manufacturer (0.5 for serum and BALF) was used [6]. However, although clinical studies of IA generally classify patients by these EORTC/MSGERC definitions, different thresholds for a positive galactomannan ODI have been used in case definitions [6]. Therefore, there is a need for further standardization of the galactomannan detection criterion.

The *Aspergillus* galactomannan group (referred to as group 3 in the main document [2]) evaluated galactomannan detection for both adults and children and its utility and clinical validity for different specimens and proposed thresholds for positivity for different clinical specimens. The group fully acknowledges that antifungal therapy is often initiated based on lower levels of evidence (based on lower thresholds for galactomannan detection) than in research settings but also felt that it was crucial to increase the likelihood of having IA in research projects. With the newly proposed thresholds we aim for high specificity (ie, to minimize the rate of false positivity) while maintaining good sensitivities, and without dramatically limiting the number of patients who would be eligible for clinical trials. As such, these proposed consensus thresholds vary from the analytical threshold that is usually recommended by the manufacturer (ie, ODI of 0.5).

BACKGROUND

Galactomannan is a polysaccharide that consists of a mannose backbone and a variable number of galactofuran side chains

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and makes up a major part of the cell wall of *Aspergillus* spp. [7]. These galactofuranose-containing polysaccharides vary in size from 35 to 200 kDa and are secreted by the fungus during growth. It is therefore an interesting biomarker to detect the presence of growing *Aspergillus* inside the human body. In May 2003, the commercially available sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia *Aspergillus* EIA; Bio-Rad, Marnes-La-Coquette, France) was approved by the Center for Devices and Radiological Health, FDA. The assay was based on the EB-A2 rat monoclonal antibody and allowed the detection of serum galactomannan. The test was approved as an adjunct for the diagnosis of IA when used in conjunction with other diagnostic procedures such as microbiologic cultures, histologic examination of biopsy samples, and radiologic evidence of disease. The test was cleared for testing of BALF in 2011.

This Platelia *Aspergillus* enzyme immunoassay is a 1-stage immuno-enzymatic sandwich microplate assay that detects all sorts of galactofuranose-containing molecules, including but not restricted to galactomannan. However, in general, the term "galactomannan" is used collectively for all molecules containing cross-reactive galactofuranose polymers.

Galactomannan is not specific for *Aspergillus* spp. as cross-reactivity with polysaccharides from closely related fungi, such as *Histoplasma capsulatum*, *Fusarium* spp., *Cryptococcus* spp., *Talaromyces* spp., *Acremonium* spp., *Alternaria* spp., *Penicillium* spp., or *Geotrichum* spp., has been described [8–12]. As a consequence, identification to the species level or detection of specific traits, such as antifungal drug resistance, is required by additional tests. Other causes of clinically significant "false positivity" result from the presence of exogenously produced galactomannan that is introduced into the body as part of another product. For example, many organic molecules such as gluconate (used in plasma expanders such as Plasmalyte Baxter) or B-lactam antibiotics (such as piperacillin/tazobactam or amoxicillin/clavulanate) are produced on an industrial scale by fermentation through *Aspergillus niger* or *Aspergillus terreus* [13]. Even after filtration, galactomannan often contaminates the final solution of this process. After oral or parenteral administration of these products, galactomannan enters the bloodstream resulting in "false positive" test results [14, 15]. Fortunately, manufacturers of these products have succeeded in reducing the amount of galactomannan in their formulations sufficiently to effectively eliminate false-positive assays [16, 17]. Food stuffs containing galactomannan (either naturally or through the addition of organic molecules produced using *Aspergillus* fermentation) can cause positive results when the permeability of the gastrointestinal barrier is increased, as is the case in intestinal graft versus host disease or severe gut mucositis [18, 19].

On the other hand, the sensitivity of the test is significantly lower in patients receiving simultaneous mold-active antifungals, either prophylactically or therapeutically [20, 21]. Moreover, sensitivity depends on the study population and the specimen [20, 22–26].

GALACTOMANNAN IN SERUM OR PLASMA

A large body of evidence supports the use of serum or plasma galactomannan detection for the diagnosis of IA, including several meta-analyses. Although testing of galactomannan in plasma has never been evaluated by the manufacturer, a postmarketing head-to-head comparison showed that the performance in plasma was equal to or better than that in serum [27]. Overall, serum or plasma galactomannan testing has a moderate to good pooled sensitivity of 0.48–0.92 and a pooled specificity of 0.85–0.95 across the different meta-analyses [20, 22, 23]. However, the diagnostic characteristics are greatly influenced by the cutoff that is being used. The assay reports an index that compares the optical density of the patient's sample with that of 2 standardized comparator samples included with the test kit. As this value is a continuous variable, different cutoffs can be selected based on the clinical scenario. For example, a lower cutoff will increase the sensitivity by picking up cases with lower values, which can be useful in a screening setting, at the cost of decreasing the specificity by also including false positives. On the other hand, if a high degree of diagnostic certainty is required—for example, in the context of a clinical trial—a higher cutoff can be chosen to increase the specificity, at the expense of lower sensitivity (Table 1). For the updated definitions, a cutoff ODI of 1.0 was selected to increase the probability of having IA compared with a cutoff ODI of 0.5 (as currently recommended by the manufacturer), as these definitions are to be used for including patients in clinical trials, where a high diagnostic likelihood is required [6]. There are even sound arguments for returning to the original threshold of 1.5 [28]; this would further increase the specificity and positive-predictive value, although at the cost of a significant reduction in sensitivity. As the conduct of clinical trials on IA is already very challenging, too high a threshold could severely limit the number of patients found eligible for enrollment. In addition, increasing the cutoff potentially induces a bias by enrolling patients with a higher fungal disease burden [4]. A serum or plasma ODI cutoff of 1.0 was therefore chosen as the best compromise between diagnostic likelihood and patient eligibility for future studies.

A second cause of heterogeneity between different studies, besides the cutoff used, is the patient population being studied. Most studies were performed in hematology patients as they are at the highest risk of IA, especially those undergoing allogeneic stem cell transplantation or induction chemotherapy for acute myeloid leukemia [29]. In this population, the sensitivity of the assay is the highest, especially when these patients are neutropenic [23]. On the other hand, the sensitivity is significantly lower in other populations that are typically not neutropenic,

Table 1. Summary of Meta-analyses of the Performance of Galactomannan in Serum or Plasma in Different Subgroups

Subgroup	Sensitivity	Specificity	PLR	NLR	Informedness
Cutoff					
0.5 ODI	0.78–0.79	0.85–0.86	5.20–5.64	0.24–0.26	0.63–0.65
1.0 ODI	0.65–0.71	0.90–0.94	6.50–11.83	0.31–0.39	0.55–0.65
1.5 ODI	0.48–0.63	0.93–0.95	6.86–12.60	0.39–0.56	0.41–0.58
Population					
HM	0.58	0.95	11.60	0.44	0.53
HSCT	0.65	0.65	1.86	0.54	0.30
SOT	0.41	0.85	2.73	0.69	0.26

Data from references [20, 22, 23]. Informedness = sensitivity + specificity – 1, also known as Youden's index or the J-statistic. Abbreviations: HM, hematologic malignancy; HSCT, hematopoietic stem cell transplantation; NLR, negative likelihood ratio; ODI, optical density index; PLR, positive likelihood ratio; SOT, solid-organ transplantation.

such as solid-organ transplant recipients and patients in the intensive care unit [23].

The performance of serum galactomannan testing appears to be largely similar in pediatric patients and adults, with a pooled sensitivity of 0.81 and a pooled specificity of 0.88 in a meta-analysis of studies in pediatric patients with cancer and hematopoietic stem cell transplant recipients [30]. However, the sensitivity of serum galactomannan testing appears to be low in patients with chronic granulomatous disease or with hyper-immunoglobulin E (hyper-IgE) syndrome (formerly Job syndrome), despite their increased risk of IA [31, 32].

GALACTOMANNAN IN BRONCHOALVEOLAR LAVAGE FLUID

Although the Platelia assay was initially only approved for use in serum, the manufacturer later also added BALF as a validated sample type. As with serum/plasma, uncertainty remains around the appropriate cutoff to be used. Overall, BALF galactomannan testing has a pooled sensitivity of 0.61–0.92 and a pooled specificity of 0.89–0.98 across several meta-analyses [24–26]. As expected, the sensitivity is highest when using the lowest ODI cutoff of 0.5, at the cost of having the lowest specificity of 0.89–0.92 as well (Table 2). Increasing the cutoff to 1.0 increases the specificity to an excellent 0.94–0.95 while only slightly lowering the sensitivity [24–26]. Further increases in the cutoff (eg, ≥1.5) only marginally improves specificity but comes with a significant decrease in sensitivity (or false-negatives) [24–26]. A cutoff ODI of 1.0 was therefore selected for the updated consensus definitions.

As with serum/plasma, the sensitivity of galactomannan detection in BALF is lower in patients exposed to mold-active antifungals [25]. Moreover, the sensitivity is negatively affected by pretreatment of viscous BALF samples with mucolytic agents [33, 34]. Unlike with serum galactomannan, the sensitivity is similar in hematology versus nonhematology patients and in neutropenic versus nonneutropenic patients [24–26].

Bronchoalveolar lavage fluid galactomannan was consistently more sensitive than serum galactomannan, both in hematology patients [26] as well as in nonneutropenic patients [35, 36]. The addition of serum galactomannan to BALF galactomannan led to a small increase in sensitivity when a positive result was defined as a positive test in either serum or BALF [26]. Therefore, the group's consensus was that the combination of 2 low positive test results, 1 in BALF and 1 in serum/plasma (BALF ODI ≥0.8 and serum/plasma ODI ≥0.7), also suggests the presence of IA, although no study has ever looked into this combination specifically.

GALACTOMANNAN IN CEREBROSPINAL FLUID

The performance in CSF was studied in a total of 42 cases of central nervous system aspergillosis [37–40]. The pooled sensitivity across these studies was 0.88 at an ODI cutoff of 0.5, 0.86 at a cutoff of 1.0, and 0.84 at a cutoff of 2.0. The pooled specificity was 0.98 across the 2 studies that used a control group and reported galactomannan values for this group, independent of the cutoff used [37, 40]. The single "false positive" in this control group was caused by a patient with a brain abscess on magnetic resonance imaging, with a CSF galactomannan ODI of 8.2, but negative culture and biopsy and no other localizations of IA [40]. He was therefore classified as not having IA in accordance with the study protocol, although this could of course also be a misclassification by the gold standard used in this study. By comparing the CSF albumin/serum albumin gradient with the CSF galactomannan/serum galactomannan, Viscoli et al [38] showed that more than 99% of the galactomannan present in the CSF of the patients was produced intrathecally, indicating that high galactomannan levels in CSF are indeed indicative of localized infection and are not just the result of translocation from the circulation. It is unclear if the performance of galactomannan in CSF is different in children. In a small study of 9 pediatric cases of cerebral aspergillosis, an ODI cutoff of 0.5 showed a sensitivity of 0.66 and a specificity of 1.00 in 32 pediatric controls [41]. Based on the aggregated data, an ODI cutoff of 1.0 was agreed upon by the group for the updated consensus definitions.

Table 2. Summary of Meta-analyses of the Performance of Galactomannan in Bronchoalveolar Lavage Fluid in Different Subgroups

Subgroup	Sensitivity	Specificity	PLR	NLR	Informedness
Cutoff					
0.5 ODI	0.82–0.87	0.89–0.92	7.45–10.88	0.14–0.20	0.71–0.79
1.0 ODI	0.75–0.86	0.94–0.95	12.50–17.20	0.15–0.27	0.69–0.81
1.5 ODI	0.70–0.92	0.95–0.98	14.00–46.00	0.08–0.32	0.65–0.90
2.0 ODI	0.61–0.84	0.95–0.96	12.20–21.00	0.17–0.41	0.56–0.80
Hematologic malignancy					
Yes	0.85	0.91	9.44	0.16	0.76
No	0.87	0.89	7.91	0.15	0.76
Antifungal therapy or prophylaxis					
Yes	0.76–0.85	0.89	6.91–7.73	0.17–0.27	0.65–0.74
No	0.91	0.88	7.58	0.10	0.79

Data from references [24–26]. Informedness = sensitivity + specificity − 1, also known as Youden's index or the J-statistic. Abbreviations: NLR, negative likelihood ratio; ODI, optical density index; PLR, positive likelihood ratio.

GALACTOMANNAN IN OTHER SPECIMEN TYPES

Besides BALF, serum/plasma, and CSF, galactomannan has also been detected in other human specimens, including urine. However, urinary antigens are expressed in different vehicles that require processing. Pilot studies have reported on the diagnostic performance of the current Platelia assay in urine using EB-A2 antibodies. These antibodies recognize long-chain galactofuranose molecules that are, however, not excreted robustly in urine [42, 43]. Using these antibodies, galactomannan could be detected in some patients, but its sensitivity was lower than in serum, despite using a lower cutoff (ODI of 0.3 or 0.1) [42, 43]. A follow-up study tried to circumvent this problem by normalizing the galactomannan ODI to urinary creatinine level [44]. In this study of only 6 cases of probable and proven IA, the urinary galactomannan to creatinine ratio had a sensitivity of 0.84 and specificity of 0.70 when using a cutoff ratio of 0.26. However, no information was provided on the performance of serum or BALF galactomannan in these same patients, making any comparison impossible.

Recently, investigators have used different antibodies that recognize shorter-chain galactofuranose epitopes, identifying fungal glycans that are expressed in animal and human urine both in free form and on the surface of extracellular vesicles [45–47]. Hence, detection of urine fungal antigen appears to be antibody dependent.

Finally, galactomannan detection in other fluids such as pus from abscesses or from suspected fungal rhinosinusitis has been described in case reports or case series [31, 48]. This appears to be useful in clinical cases where other tests were not helpful but has not been validated on a larger scale. As there are insufficient evidence and experience with all of these specimen types to date, they were not included in the latest revision of the consensus definitions of IA.

NOVEL *ASPERGILLUS* ANTIGEN TESTS

Following the most recent consensus meeting in 2015 (which resulted in the second revision and update of the EORTC/MSGERC definitions [3]), novel *Aspergillus* antigen detection tests have been investigated. Recently, the commercially available IMMY lateral flow assay (IMMY, Norman, OK, USA) and the OLM Diagnostics lateral flow device (OLM Diagnostics, Newcastle Upon Tyne, United Kingdom) have been approved for use as a diagnostic aid. These are fast and effective alternatives to galactomannan detection and prove to be especially useful for centers with low sample throughputs [49]. More recently, a lateral flow dipstick assay using the galactofuranose-specific monoclonal antibody (mAb476), which recognizes urine antigens after *Aspergillus* fumigatus pulmonary infection in animals, demonstrated good sensitivity and specificity, especially in patients with cancer [46]. This assay and an enzyme immunoassay are currently undergoing a multicenter clinical

validation. In addition, several new competing assays are under development by companies such as Dynamiker, Euroimmun, and IMMY, but large-scale data are lacking so far. Importantly, performance of the new tests in detecting fungal antigens in different body fluids will likely differ from the current Platelia galactomannan assay based on antibody epitope recognition (as discussed in urine) and test format. The group has decided not to incorporate these tools as a microbiologic criterion in the updated definitions as their performance as well as the corresponding cutoffs have not yet been fully assessed.

IMPACT OF NEW CUTOFFS

As discussed, we recommend these new cutoffs in order to increase the specificity of identifying cases, an important goal for enrollment in antifungal treatment trials. The group recognizes that our recommendation may impact clinical studies and clinical scenarios differently, depending on the focus. For instance, use of higher cutoffs to identify cases may not be feasible in the context of prophylaxis or prophylactic studies, where clinicians may want to treat patients with evidence of disease and antigen levels meeting the manufacturer's recommended lower cutoff. Adjustment of the cutoff has the potential secondary effect of changing the relevance of the "possible" IA category, with particular impact in prophylaxis and diagnostic studies, as a larger proportion of these patients can be considered to have real disease.

CONCLUSIONS

As the goal of the EORTC/MSGERC consensus definitions is to facilitate standardization and the selection of a more homogeneous population of patients with IA for clinical treatment trials, the proposed galactomannan cutoffs are higher than those typically used in clinical care. This results in a higher specificity and diagnostic likelihood, at the expense of a slightly lower sensitivity. In the end, the cutoffs that are being proposed are based on a consensus decision on the optimal tradeoff between diagnostic certainty and ensuring that a sufficient number of patients remain eligible for enrollment in treatment trials. It is important to note that all cutoffs mentioned in these consensus documents are based on the Platelia *Aspergillus* assay. We hope that the application of the new criteria in clinical, diagnostic, and epidemiologic research of IA will result in further standardization and improved comparability.

Notes

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