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REVIEW PAPER

Prognostic value of galactomannan: current evidence for monitoring response to antifungal therapy in patients with invasive aspergillosis

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Abstract Galactomannan (GM) is a polysaccharide present in the cell wall of *Aspergillus* spp. that is released during growth of the organism. It has been successfully used to aide in the diagnosis of invasive aspergillosis allowing for earlier recognition of disease compared to conventional methods. Since its implementation in the clinic as a diagnostic tool, GM has been used in experimental models to measure therapeutic response. Several clinical studies describe the prognostic value of GM. Herein, we review the evidence supporting the utilization of GM antigen as a biomarker to measure response to systemic antifungal therapy.

Keywords Galactomannan · Biomarker · Aspergillosis · Antifungal · Prognostic tool

Introduction

Invasive aspergillosis (IA) causes significant morbidity and mortality [1]. Treatment is complicated by the limited number of antifungal classes and agents, antifungal drug toxicity, an incomplete spectrum of antifungal activity, and increasing incidence of antifungal resistance [2–6]. The routine management of patients is usually complicated by uncertainty in the diagnosis, delayed treatment and absence of information on the appropriate duration of therapy. One of the biggest hurdles in clinical care is the assessment of the response to therapy at the bedside. Clinical signs and symptoms are notoriously nonspecific, radiological abnormalities often worsen before improving and clinical response to therapy occurs over weeks-to-months. Better clinical and laboratory tools that enable the therapeutic response to therapy to be followed would be a considerable advance in the routine care of patients with IA. In addition, objectively assessing clinical outcomes in therapeutic trials of new antifungal drugs is difficult and could be improved with the use of a biomarker such as galactomannan (GM).

Galactomannan is a polysaccharide present in the cell wall of *Aspergillus* spp., and some other fungi [7]. It is released by hyphae, which are the biologically invasive forms of filamentous moulds. A commercial double-sandwich enzyme-linked immunosorbent galactomannan assay (ELISA) is available to detect *Aspergillus* GM antigen in body fluids. The assay uses a monoclonal IgM obtained from rats following challenge with mycelial extracts from *Aspergillus* spp. [7, 8]. The assay has become a standard of care for the diagnosis of invasive disease in high risk patients [9].

Herein, we review the literature on the use of GM as a biomarker of therapeutic response both in experimental models and in the treatment of patients infected with *Aspergillus* spp. A review of the literature was completed using a standard PubMed search for "galactomannan and pharmacodynamics", "galactomannan and *Aspergillus* spp.", "triazole and galactomannan", "echinocandin and galactomannan", and "polyene and galactomannan". Articles related to the use of GM as a marker of response to treatment were reviewed.

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Structure and biology of galactomannan

Galactomannan is a polysaccharide cell wall component that has a branched structure with a linear α mannan that has a repeating mannose oligosaccharide unit [6Manα1–2Manα1–2Manα1–2Manα1] and short chains of β (1,5) galactofuranose residues [7]. It is released from fungal hyphae during growth in the extracellular material (i.e. ethanol-precipitable material) as part of the chemical breakdown in the cell as the hyphae grow (i.e. a metabolic event) and not a result of mycelial lysis. Organisms that are known to release GM include *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., *Alternaria* spp., *Histoplasma* spp. and *Penicillium* spp. [10–14]. GM can be detected in serum and other bodily fluids such as bronchoalveolar lavage (BAL) and cerebrospinal fluid (CSF), and has been used to support a diagnosis of IPA and cerebral aspergillosis, respectively. There are no established cut-offs values for GM from matrices outside of the serum.

Aspergillus releases GM as part of recycling process of the cell wall during hyphal growth. GM measurement is achieved by using sandwich enzyme-linked immunosorbent assay (ELISA), which detects galactomannan antigen via binding to monoclonal antibody and the formation of a monoclonal antibody-galactomannan-monoclonal antibody/peroxidase complex. A spectrophotometer is used to determine the absorbance (optical density; OD) of the samples. Serum samples are considered positive when the GMI (OD) is $\geq$0.5 and negative when <0.5. GMI has no units and if expressed as a ratio of the OD value of the sample to the OD value of a standard sample containing 1 ng of GM. The test has a lower limit of detection of 1 ng/mL. GM is utilized primarily as a diagnostic test; however, evidence is increasing for its value as a prognostic test.

How is GM linked with the pathogenesis of IA

The release of GM correlates with the progression of disease and its resolution in experimental models of IA [15]. Figures 1 and 2 shows how GM levels change over time in rabbits (Fig. 1) [16] and humans (Fig. 2a, b) (submitted for publication). Clear trends are seen when an infection responds to effective therapy. Levels of GM in the serum correspond to the timing of tissue invasion and are an integral measure of the mass of viable invading organisms. GM can be detected as early as 12 h post inoculation, which corresponds with hyphal invasion as determined using histopathological techniques [15, 17]. Serum GM levels continue to increase and eventually reach a plateau as the ELISA assay is saturated and/or there is capacity limitation of fungal growth following exhaustion of available nutrients. BAL GM concentrations are typically high throughout the course of infection in experimental models.

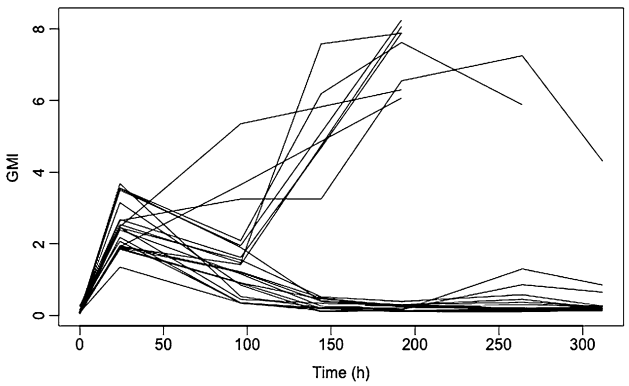

Fig. 1 Galactomannan index over time in rabbits treated in an experimental model of invasive pulmonary aspergillosis infection [16]

Presumably this reflects hyphal growth in the airways and does not necessarily imply invasion. The presence of GM implies the presence of hyphae rather than conidia (the latter do not liberate GM) and in this sense GM provides slightly different information than culture and PCR from samples from the airways, which may not necessarily distinguish the different fungal morphotypes.

The discordant kinetic profiles of GM in serum and BAL suggest that the GM does not transverse the alveolar-capillary bilayer or other biological barriers to any great extent. Presumably its molecular weight ($\sim$20 kDa) [7] prevent transgression even in the context of significant tissue disruption. The appearance of GM in the circulation therefore represents angioinvasion rather than simple diffusion into the bloodstream from contiguous areas of infection.

To describe the changes in GM after infection and after exposure to systemic antifungal therapy, several PK–PD mathematical models have been described. One such example, a version of a logistic growth model, is provided below:

$$\frac{dX(1)}{dt} = Kg \cdot \left(1 - \frac{X(1)}{POPMAX}\right) \cdot X(1) \quad (1)$$

where Kg represents the growth constant and POPMAX represents the theoretical maximum GM concentration. Here, as the GM concentration reaches the maximum value, growth decreases until eventually ceasing. To extend this to include population PK modeling for the antifungal, the following example equation has been used to describe the relationship of the two together:

$$\frac{dX1}{dt} = -Ka \cdot X1 \quad (2)$$

$$\frac{dX2}{dt} = Ka \cdot X1 + RateIV - \frac{V_{max}}{K_m \cdot V + X2} \cdot X2 - Kcp \cdot X2 + Kpc \cdot X3 \quad (3)$$

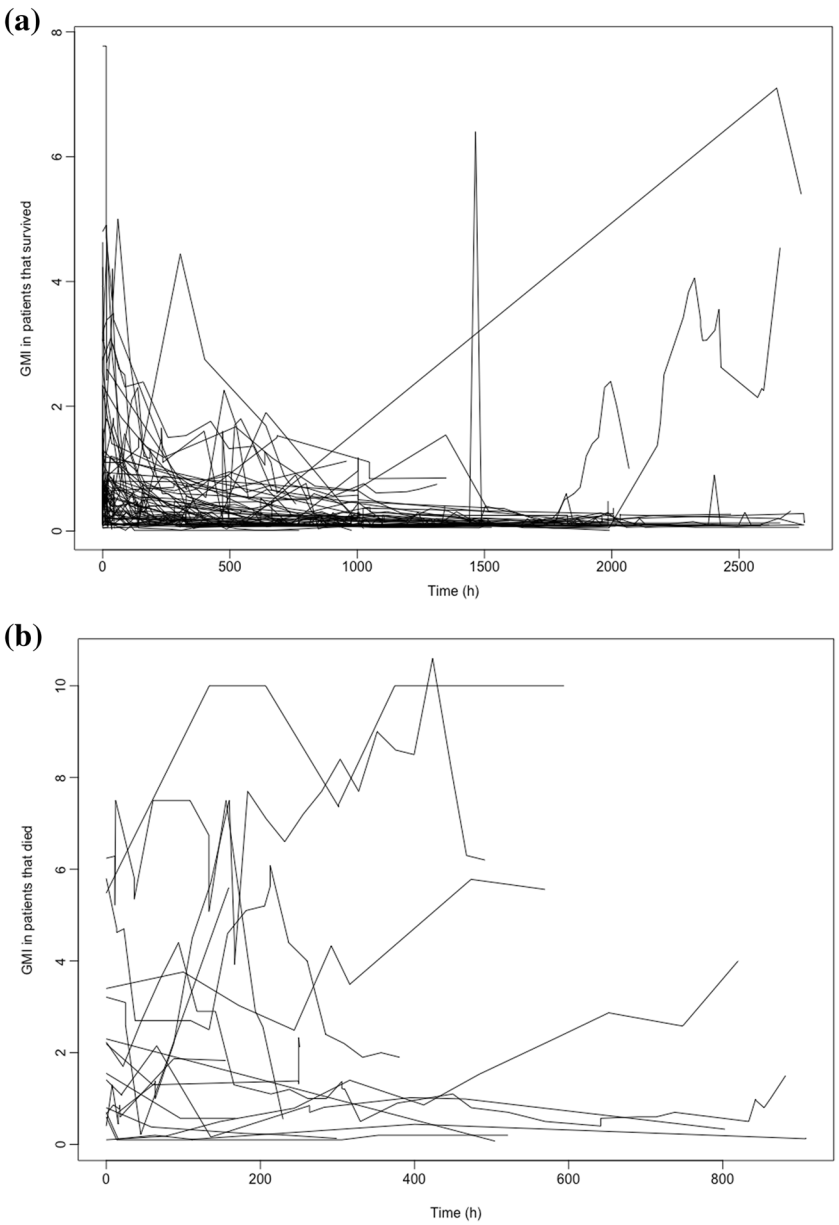

Fig. 2 Observed galactomannan index values over time in patients with invasive aspergillosis who were alive after 42 days of treatment (**a**) and those patients that did not survive to day 42 (**b**), (data on file)

$$\frac{dX3}{dt} = Kcp \cdot X2 - Kpc \cdot X3 \tag{4}$$

$$\frac{dX4}{dt} = KGM_{prod} \cdot \left[1 - \left(\frac{X4}{POP_{max}}\right)\right] \cdot \left(1 - \frac{\frac{X2^H}{V}}{EC_{50}^H + \frac{X2^H}{V}}\right) \cdot X4 - KGM_{elim} \cdot X4 \tag{5}$$

where Eqs. 2–4 describe the flow of drug and Eq. 5 describing the relationship of drug and galactomannan production and elimination.

Early in vivo studies suggest that the major pathway for *Aspergillus* GM clearance from the bloodstream is renal excretion and hepatic metabolism based on uptake by macrophage mannose receptors [18]. The majority of the renal excretion is rapid, within the first 24 h. Detectable of GM in the urine of patients with IA has been reported [19].

GM is a complementary measure of fungal burden in experimental models of IA

The traditional measure of tissue burden obtained by plating serial dilutions of tissue homogenates to agar does not (in general) provide a useful measure of fungal burden. There are several reasons for this. In some experimental models, sampling at early time-points may not enable conidia (environmental forms) that are used to initiate infection to be distinguished from hyphae (tissue invasive forms). Detection of both morphotypes leads to a positive

culture or PCR signal and quantification of fungal burden even though it is only hyphae that are biologically relevant in this situation. An accurate quantification of fungal burden is further complicated by the growth patterns of *Aspergillus*, which extends via hyphal elongation with indistinguishable cellular units and additional branching from older and newer segments [20]. Tissue homogenization and quantitative counts do not enable an accurate estimate of the fungal biomass. Propagules are either incompletely separated or more vigorous grinding leads to complete hyphal disruption and subsequent fungal death. In either situation $\log_{10}$CFU/g is an inaccurate reflection of the underlying fungal biomass and neither reflects important events in the pathogenesis nor response to antifungal therapy.

Despite these potential limitations, fungal burden ($\log_{10}$CFU/g) from the rabbit model provides a crude, but reliable readout to assess a variety of antifungal agents (with the possible exception of the echinocandins). In this model sampling occurs late in the treatment period and is probably not confounded by the presence of conidia. In other model systems, $\log_{10}$CFU/g is completely non-informative. In any context $\log_{10}$CFU/g is often too imprecise to construct detailed dose–exposure–response relationships.

Other non-culture techniques used to estimate fungal burden in experimental models after infection with *Aspergillus* spp. include polymerase chain reaction (PCR) to measure of the amount of fungal DNA in tissues, or assays that measure the amount of fungal cell wall components such as chitin and 1,3-β-D-glucan. Each method has advantages and limitations (see Table 1).

GM and experimental pharmacodynamics

GM is increasingly used in pharmacodynamic models of IA to estimate dose-exposure-response relationships. The advantages of this biomarker include a rapid response to antifungal therapy, the fact it is readily quantifiable, the availability of a commercial kit and validation in a variety of model systems. The disadvantages include a relatively narrow dynamic range, relatively high expense, and large inherent variability.

Does serial GM kinetics and response differ by drug class?

GM kinetics may differ according to drug class. With the possible exception of the echinocandin agents, a decline in GM occurs in a drug exposure-dependent manner [16, 21–24]. Circulating GM may be paradoxically higher following exposure of *Aspergillus* to all three of the licensed echinocandin agents (i.e. caspofungin, micafungin and anidulafungin). Combination with other antifungal agents may be necessary to elicit a sufficient and sustained decline in GM index (GMI) [25–27]. This paradoxical effect is probably a result of the unique mechanism of action of the echinocandins, which induce grossly aberrant

Table 1 Comparison of experimental techniques to measure *Aspergillus* fungal burden

Method	Description	Advantage	Disadvantage
CFU	Counting of single organisms by plating of serial dilutions of a suspension; using a hemacytometer	Simple, inexpensive	Homogenation by mechanical dispersion can disrupt the count by breaking the hyphae into smaller pieces causing either fewer viable fragments or increase in viable units
Chitin-assay	Measure of the amount of chitin by KOH extraction and colorimetric assay of an aldehyde derivative of chitosan	Measuring content in hyphae not conidia since chitin is not present in conidia	Does not distinguish between viable and non-viable hyphae, nor viable but ungerminated conidia can go undetected.
PCR	Measure either 18S rDNA (present in the genome in 100 per nucleus) or *FKS1*, single-copy gene	Better accuracy than CFU depending on the tissue especially in the first few days after inoculation	Does not distinguish between viable and non-viable organisms. Use of 18S rDNA can over-estimate fungal burden given the multinucleic nature of *Aspergillus* spp. High cost, complex
Lateral-flow device	Detects the presence of *Aspergillus*-specific MAb (JF5) [58]	Rapid detection of the presence *Aspergillus* spp. within an immune-chromatographic lateral-flow device. Measure of growing *Aspergillus*	No quantitative details of the amount of organisms
Electronic nose	Detects volatile organic compounds (VOCs) using an "electronic nose" which is an artificial olfactory system to discriminate odors using an array of sensors [59]	Rapid detection of presence of *Aspergillus* spp. in the airways	No quantitative details of the amount of organisms Only pertinent to pulmonary infection

CFU colony-forming units, *KOH* potassium hydroxide, *PCR* polymerase chain reaction, *rDNA* recombinant deoxyribonucleic acid

branching of hyphae that impairs the ability of the organism to invade tissues. The organism is not killed, but changes to the cell wall result in excessive liberation of galactomannan. Thus there is de-linkage of the PK–PD relationships when GM is used to assess the response to echinocandin therapy.

A decline in GM in rabbits infected with *A. fumigatus* after treatment with triazole antifungal agents is not evident until after 4 to 6 days of treatment. In contrast, polyenes demonstrate a more rapid response within the first two days of therapy [21, 22, 28]. There are further idiosyncrasies related to the various amphotericin B formulations. The dosage (on a mg-mg basis) required to induce a decline in GM is strikingly different amongst the clinically licensed compounds (i.e. liposomal amphotericin B (LAMB) > amphotericin B lipid complex (ABLC) > amphotericin B deoxycholate (DAmB)) [29]. This is likely a function of the thermodynamics that governs the transfer of active drug from the micelle (DAmB) or lipid carrier (ABLC and LAmB).

Do GM changes differ by *Aspergillus* species?

Studies evaluating non-*Aspergillus fumigatus* or *A. fumigatus* with CYP51A mutations indicate that there are differences among species with regard to response to therapy and behavior in the model that limit generalizations across *Aspergillus* species. A decline of GM is most dramatic with *A. fumigatus*, followed by *A. flavus* and *A. terreus* [30–32]. In *A. terreus*-infected animals, the rate of increase of GM post inoculation are slower than that of *A. fumigatus* [33]. This probably reflects significant differences in the rate and extent of germination in the first 24 h in *A. terreus* compared to *A. fumigatus*. In the first 8 h, less than 1.5% of the *A. terreus* conidia germinate compared to 97% of *A. fumigatus*.

The minimum inhibitory concentration (MIC) generally affects exposure response relationships [34–39]. This has been demonstrated repeatedly in pharmacodynamic studies of triazole agents when testing multiple organisms over a range of MICs [36, 40, 41]. Even in the presence of mutations in the cellular target, response can be demonstrated when exposures above the PD target are achieved and this is can be predicted by the MIC values of the organisms.

GM as a clinical biomarker with prognostic value

Clinical evidence supporting the utility of GM as a prognostic tool for patients with IA has steadily accrued over the past two decades. The earliest suggestion that GM might be a valuable biomarker for measuring therapeutic response in patients was established in 1997. However, the general utility of exploiting the prognostic value of GM was initially limited by the relatively high number of false positive results [42]. In addition, early case reports describe increasing GMI during treatment in individual patients with poor outcomes. [43–45].

Evidence from case series and clinical studies

The first clinical study conducted early in the use of GM was limited by sparse sampling. However, a trend of increasing GM in patients that subsequently died was evident [42]. Subsequently, nine separate studies representing 661 patients and one meta-analysis covering 27 studies and 257 patients that evaluated the utility of GM assay to monitor therapeutic response have been reported. Eight studies largely focused on patients with hematological malignancy, while one included patients with COPD. The studies evaluate responses in patients with proven, probable, possible, or suspected IA and one study included patients with invasive fusariosis. One study assesses the use of serial GM in pediatric patients and the associated exposure-response relationship. Each study demonstrates the potential usefulness of serial GM measurements in IA patients by showing that GM generally increases (or does not decline) in patients that fail treatment or ultimately die.

Correlation with traditional measures of response to therapy

Changes in GMI from the time of diagnosis or baseline correlate significantly with various outcome measures including clinical outcome defined by EORTC/MSG criteria at 6 and 12 weeks, mortality at 12 weeks, and with autopsy findings [46–49]. The GMI-based outcome proposed from these correlations is defined as GMI negativity (OD < 0.5) for at least 2 weeks after the first positive value without new pulmonary or extra-pulmonary lesions and lack of findings of IA on autopsy [46, 47]. Figure 3 provides an illustration of survival trends for neutropenic patients with positive (OD ≥ 0.5) versus negative (OD < 0.5) GMI.

GMI-based outcome criteria—are we there yet?

Several outcome measures for GM are possible and include time to GMI negativity, time to a certain percent reduction in GMI, rate of decline (or GM decay), area under the GMI time curve, or time to negativity. However, with all of these possibilities, a degree or quantification of GMI change aside from negativity has yet to be defined. The only criterion that is currently used is GMI negativity (OD < 0.5) for a period of 2-weeks without pulmonary or extra-pulmonary lesions. An autopsy may be necessary to

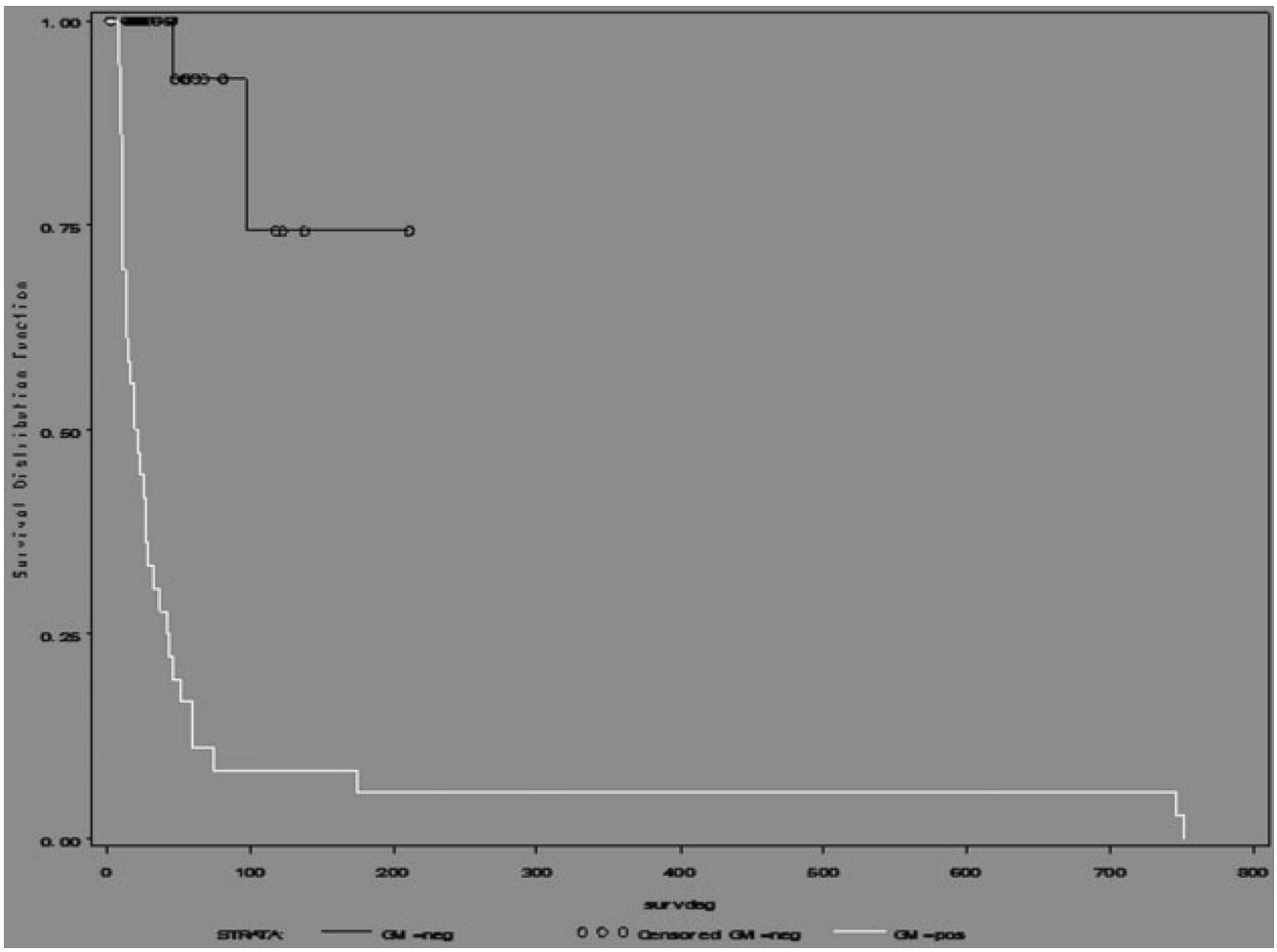

Fig. 3 Adapted from Maertens et al. [46] demonstrating the survival of 70 neutropenic patients with IA according to serum GMI

definitively exclude IA. While this GM outcome provides excellent correlation to clinical measures of response, the average time to negativity in one study was 21 days in patients who ultimately responded to antifungal therapy. An earlier intervention may be preferable [47]. The rate of decline of GM (i.e. slope) at 1 week after diagnosis of IA has also been proposed as a surrogate for clinical outcome (i.e. for 6- and 12-week all-cause mortality) based on increasing hazard ratios with each unit increase from diagnosis [50]. This GM outcome measure is more practical than the GM outcome of negativity for 2 weeks duration as it utilizes an early time point and relatively simple calculation to guide response.

Significant increases in GM after each week of therapy are associated with ultimate treatment failure [51–53]. Most studies report a strong correlation of survival with decreases GMI (e.g. GMI normalization to readings <0.5) [46, 48]. However, at least one study reports that changes from baseline to week 2 are not predictive of 12-week survival [52]. The reason is most likely because mortality by 12 weeks is more often driven by underlying co-morbidities as opposed to the fungal infection.

Other fungal biomarkers, such as, the 1,3-β-D-glucan (BDG) assay (Fungitell^{TM}), are used in the clinic. BDG detects the cell wall component (1,3)-β-D-glucan, which is present in most fungi, and therefore, not specific to *Aspergillus* spp. This assay could potentially be used in conjunction with GM as a prognostic tool after IA is diagnosed. However, there is little data available showing correlation to clinical outcome outside of experimental models [17, 54]. One study suggests that decline in the mean time-weighted averages of BDG plus GM from baseline to week 2 of therapy is associated with 6-and 12-week survival [53].

Using GM to individualize antifungal therapy

The time course of GM in an individual patient may be affected by antifungal drug exposure (pharmacokinetics), the MIC of the invading fungal pathogen, the immune

status of the host, and the underlying high fungal burden (pharmacodynamics). Therapeutic drug monitoring of antifungal agents has traditionally focused on achieving plasma drug exposure targets. The pharmacodynamic responses to antifungal therapy have typically been less formal. However, GM can potentially be used to guide antifungal therapy at an individual level. The time course of GM may provide a guide as to the intensity of antifungal therapy that is required to achieve a favorable clinical outcome. Patients with high unremitting GM concentrations receiving a standard antifungal regimen should have the dosage increased, the drug changed or a combination of agents used. The necessary mathematical models that explicitly link dosage, plasma drug concentrations and circulating GM provide a way that therapy can be individualized to move the biomarker rather than merely achieving a target plasma concentration, which may not be optimal for the patient.

A further possibility that is enabled by GM is an estimate of the pharmacodynamic targets required for a successful outcome. Traditionally, this pharmacodynamic measure has been the MIC and drug exposure (e.g. AUC) has been optimized with reference to this in vitro measure of potency. A common problem in clinical mycology is that the organism (and therefore the MIC) is not available-it is quite uncommon for patients to have positive cultures. The use of linked PK–PD models with GM as a real-time pharmacodynamic readout provides alternative measures of in vivo potency that can be used to optimize drug exposure. The EC_{50} is the concentration of antifungal drug that is required to induce half maximal antifungal activity. The AUC can be optimized in relation to the EC_{50} to secure a favorable outcome. This idea was explored in a recent relatively small study in pediatric patients receiving voriconazole [55]. In these children an $(AUC:EC_{50})/15.4$ is significantly associated with terminal GMI (GMI value at the end of therapy). When the ratio is >6, the terminal GM tends to be lower. However, survival did not correlate with the $AUC:EC_{50}$. Further work is ongoing in this area.

Evidence for GM beyond *Aspergillus* spp. and hematological malignancies

Moulds other than *Aspergillus* spp. also release GMI. One study describing 18 patients diagnosed with invasive fusariosis shows a median time to GMI negativity of 14 days from the first positive GMI [56]. However, there was no relationship between the time to GM negativity and the 90-day outcome. In non-hematology patients, data for the use of GMI as a prognostic marker is sparse. One study in COPD patients showed that in non-survivors GMI is higher (>0.5) in the first week [57], but there was a very high rate of false positive results. There remains a paucity of evidence for the use of GMI to follow the course of disease beyond *Aspergillus* spp. and outside the hematological malignancy population.

Conclusions

GMI is an important experimental and monitoring tool in the management of patients with IA. Critical steps to establish the clinical utility of this biomarker continue to be made. However, more work is needed in patients to better define ways of using GMI early in the treatment course to facilitate therapeutic decisions that can be beneficial for an individual patient. As evidence accumulates, it is likely that GM will be incorporated into clinical outcome criteria and can therefore be used to assess the response to antifungal therapy for future clinical trials.

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