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Serum Galactomannan and (1→3)-β-D-Glucan Assays for Patients with Multiple Myeloma and Waldenstrom's Macroglobulinemia

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We assessed the performance of galactomannan and (1→3)-β-D-glucan in 29 serum samples from patients with multiple myeloma and Waldenstrom's macroglobulinemia without invasive fungal disease to address issues of false positivity and uninterpretable results previously reported among patients with these conditions. Galactomannan and (1→3)-β-D-glucan assays were not falsely elevated in any patient. (1→3)-β-D-glucan assay results were uninterpretable in 24% of patients. Patients with IgG levels of >2,000 mg/dl had higher odds of uninterpretable (1→3)-β-D-glucan results.

Invasive fungal disease (IFD) is a significant cause of morbidity and mortality in patients with hematologic malignancy (9, 14, 15, 19). Multiple myeloma (MM) and Waldenstrom's macroglobulinemia (WM) are plasma cell diseases associated with increased monoclonal serum immunoglobulin levels. Serum galactomannan (GM) and (1→3)-β-D-glucan (BG) antigen assays are useful for the diagnosis of invasive aspergillosis and other IFD in patients with appropriate risk factors and a compatible clinical syndrome (2–7, 10–13, 16–18). MM and WM patients may have uninterpretable GM or BG assay results, presumably due to optical interference from high levels of paraprotein (20), potentially making these assays less useful in this population. Other known causes of optical artifacts when using the BG assay include excessive hemolysis, hyperbilirubinemia, and lipemia. In one study, GM was reported to be falsely elevated in up to 50% of patients with immunoglobulin G (IgG)-subtype MM (8). We conducted this study to assess the performance of GM and BG assays in MM and WM patients without IFD, evaluate the rate of false-positive results, and identify potential factors associated with uninterpretable results.

TABLE 1 Patient characteristics

Characteristic	Value
No. of patients	29
Median age, years (range)	63 (41–88)
Sex, no. (%)	
Male	14 (48%)
Female	15 (52%)
Race/ethnicity, no. (%)	
White	27 (93%)
Nonwhite	2 (7%)
Underlying disease, no. (%)	
Multiple myeloma	17 (59%)
Waldenstrom's macroglobulinemia	11 (38%)
Solitary plasmacytoma	1 (3%)
Immunoglobulin type	
IgG	11
IgM	10
IgA	1
Light chain	5
Nonsecretory	2
Immunoglobulin levels, mg/dl; median (range)	
Overall	
IgG ($n = 29$)	896 (109–4,050)
IgM ($n = 29$)	45 (2–5,080)
Multiple myeloma ($n = 17$)	
IgG	1990 (896–4,050)
IgG > 2,000 mg/dl ($n = 4$)	2,840 (2,560–4,050)
Waldenstrom's macroglobulinemia ($n = 12$)	
IgM	1,700 (57–5,080)

Serum samples were obtained from MM and WM patients without clinical or radiologic signs of IFD who presented to Dana-Farber Cancer Institute in Boston, MA, between November 2010 and January 2011. Serum samples were tested using commercially available GM (Platelia; Bio-Rad Laboratories, Hercules, CA) and BG (Fungitell; Associates of Cape Cod, East Falmouth, MA) assays by technicians blinded to patient characteristics and sample immunoglobulin type and levels. Pertinent clinical data, including patient demographics, MM type, and Ig levels, were recorded. All analyses were performed using STATA 11 (College Station, TX). Logistic regression was used to assess factors increasing the odds of an uninterpretable BG value.

To investigate the effect of BG assay buffer pH in the generation of potential optical artifacts, serum samples from patients with high IgG levels (>2,000 mg/dl) were incubated at different pH levels (6.5 to 8.0) and assessed for paraprotein precipitation and development of optical artifacts. Samples were processed using the following protocol: 5 μl of serum was preincubated with 20

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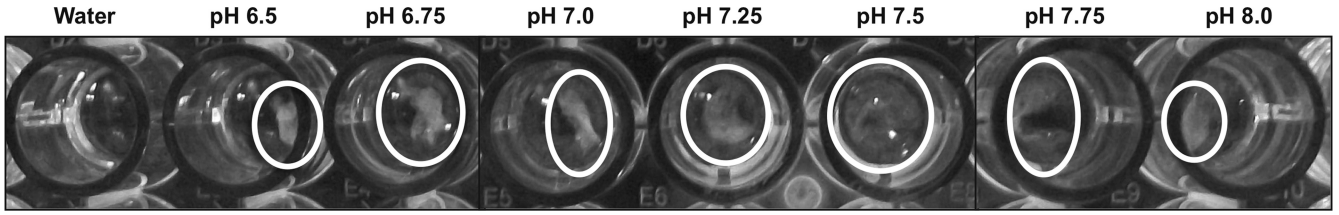

FIG 1 Incubation of a representative serum sample with IgG levels of >2,000 mg/dl at different pH levels (6.5 to 8.0). White precipitate is visible in all but the first well, in which water was used as the diluent.

μl of an alkaline pretreatment reagent (0.125 M KOH, 0.6 M KCl) at 37°C, and then 100 μl of a 0.1 M Bis-Tris propane buffer at a range of pH values (6.5 to 8.0) or water was added to this mixture. Optical density was read at A_{405} minus A_{490} using a Molecular Devices ThermoMax microplate reader (SoftMax Pro v3.1.1). Each experiment was conducted in quadruplicate. This study was approved by the Office for Human Research Studies at Dana-Farber/Harvard Cancer Center.

Serum samples were obtained from 29 MM and WM patients. Baseline characteristics, immunoglobulin class, and levels are presented in Table 1. All serum samples tested negative for GM, with a median GM index of 0.17 (range, 0.12 to 0.30).

A total of 22 (76%) BG assays were negative, with BG values of <31 pg/ml. A total of 7 samples (24%) had uninterpretable BG assay results due to optical artifacts, including four IgG-type MM, one IgM-type WM, and two light-chain MM. Of 4 samples with IgG levels of >2,000 mg/dl, 3 had an uninterpretable BG assay result. On univariable logistic regression, an IgG level of >2,000 mg/dl, was the only factor predictive of an uninterpretable BG result (odds ratio, 7.5; 95% confidence interval [CI], 0.9 to 60.4; $P = 0.05$). Sample turbidity, total protein, bilirubin, and IgM levels were not predictive of uninterpretable BG results.

Precipitation was observed when BG assay buffer was added to serum from 3 patients with IgG levels of >2,000 mg/dl across a wide pH range (6.5 to 8.0). In contrast, precipitation was not observed when water was used as a diluent (Fig. 1). There was no correlation between pH and change in the mean velocity (V_m) of optical density of the BG assay (Fig. 2).

In contrast to a previous report in which 50% (11 out of 22) of patients with IgG-type MM had false-positive GM assay results (8), we did not find any falsely elevated GM in our cohort of patients with MM and WM. Although we found no false-positive BG results, BG results were not interpretable due to optical artifacts in 24% of samples, likely due to paraprotein precipitation.

The Fungitell BG assay relies on the activation of the BG-sensitive zymogen proteases of the reagent, with hydrolysis of a chromogenic substrate (leucine–glycine–arginine–para-nitroaniline) and an increase in the A_{405} optical density. The final assay result is based upon the mean velocity (V_m) of the rise in optical density over the 40-min test period (Fungitell test procedure outline: http://www.acciusa.com/clinical/fungitell/FungitellInfoDownloads.html). The pattern of optical density development over the incubation period of the assay offers an opportunity to observe potential optical artifacts. In cases with optical artifacts, the optical density usually rises immediately, without the initial lag period associated with zymogen protease activation (Fig. 3).

MM patients with IgG levels of >2,000 mg/dl had higher odds of uninterpretable BG results secondary to optical artifacts. While IgA and IgM have isoelectric points in the acidic range (pH 4.5 to 6.5), IgG has an isoelectric point in the basic range (pH 6.5 to 9.5) (1). As the BG assay buffer has a pH of 7.4, it is possible that samples with high IgG levels precipitate, since they experience a pH level close to their isoelectric point. However, changing the BG assay buffer pH (range 6.5 to 8.0) had no effect on paraprotein precipitation, and only samples with a water diluent had no observed precipitation. There was also no correlation between pH and V_m of optical density (Fig. 2). The observed variation in V_m was most likely due to paraprotein precipitation randomly interfering with the optical density reading. Whether optical interference effect is limited to monoclonal Ig needs to be further studied.

In summary, GM and BG were not falsely elevated in patients with MM or WM and can be used in patients with suspected invasive fungal disease. Patients with plasma cell disorders and

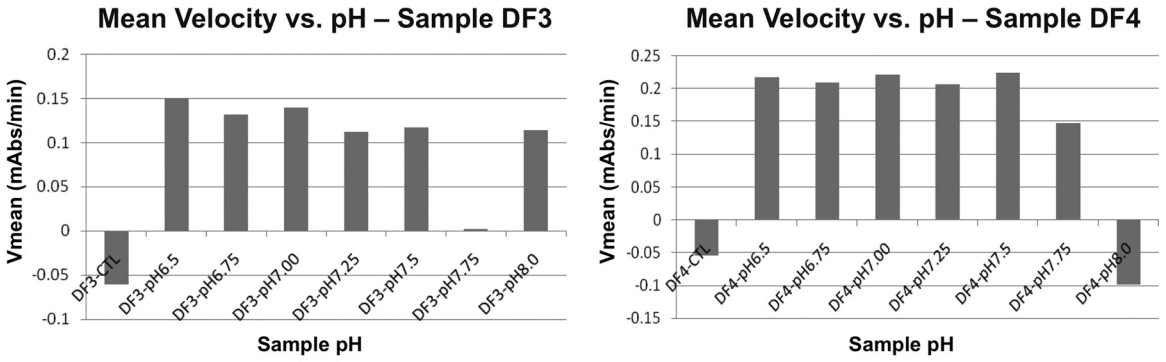

FIG 2 Correlation between pH and V_m of optical density in two representative samples with IgG levels of >2,000 mg/dl. CTL, negative control; V mean, mean velocity; mAbs, milliabsorbance units.

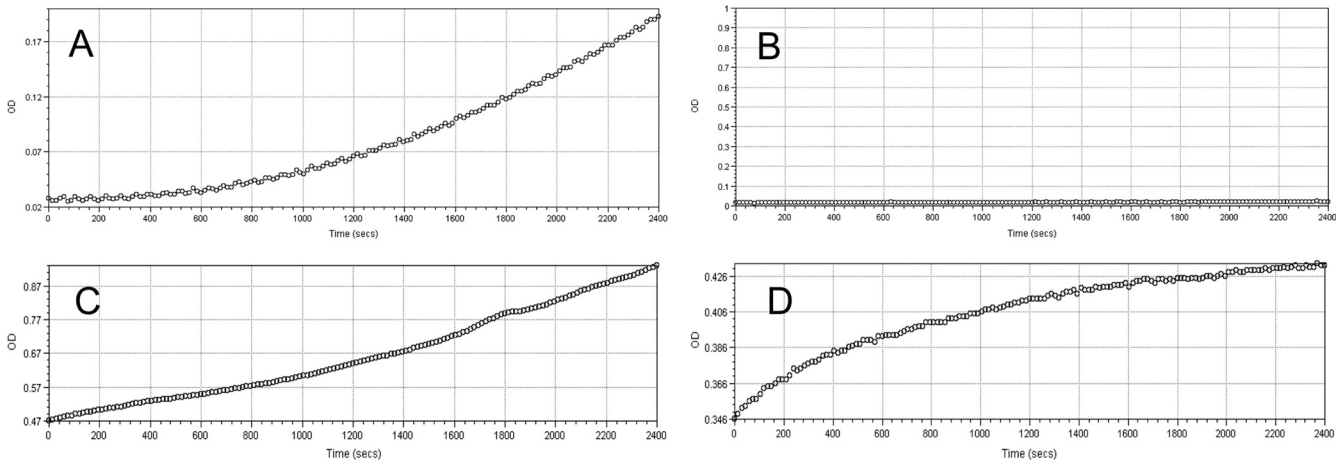

FIG 3 (A) BG assay kinetic trace of a positive sample without optical artifacts; (B) kinetic trace of a negative control; (C and D) kinetic trace showing optical artifacts. OD, optical density. In panels C and D note initial high OD readings and the absence of initial lag periods consistent with optical artifacts.

IgG levels of >2,000 mg/dl have higher odds of uninterpretable BG results.

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