

Measurement of (1-3)- β -D-Glucan Derived from Different Gauze Types

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(1-3)- β -D-glucan (BDG) is a cell-wall polysaccharide component found in most fungi. The measurement of BDG is a useful diagnostic marker for invasive fungal infections. However, it is well known that interfering substances can result in false positive reactions. We encountered a patient who underwent lung transplantation and presented with highly elevated BDG values, despite having no evidence of invasive fungal infection. We therefore hypothesized that elevated BDG values were originated from the gauze products used during surgery. While it is known that gauze products contain BDG, there have been no previous reports to quantitatively correlate amount of gauze usage and BDG levels. In this study, we extracted BDG from various gauze products and measured BDG to better understand the degree of which gauze contributes to elevated BDG values. Six types of commonly used surgical gauze products were selected for our study. Each of the surgical gauze was immersed in sterile, purified water for up to 120 minutes. At set intervals, BDG values in the water extracts were measured. Purified water samples without gauze were used as negative controls (< 4 pg/ml). After 120-minute extraction, BDG levels varied greatly depending on gauze products, ranging from 11.7 pg/ml to 6612 pg/ml. The gauze made of lyocell, which is a fiber produced from wood pulp cellulose, yielded the lowest levels of BDG, and probably would not cause false positive for fungal infections. There is a need for the development of a gauze product that does not contribute to elevated BDG values. ——— gauze; surgical operation; (1-3)- β -D-glucan; false positive value.

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The diagnosis of invasive fungal infection continues to be a challenge to the clinician as current laboratory methods are limited in usefulness. Of the non-culture methods of diagnosis, detection of (1-3)- β -D-glucan (BDG) has received attention as a useful aid in rapid diagnosis of invasive fungal infection (Obayashi et al. 1995; Ostrosky-Zeichner et al. 2005; Pickering et al. 2005). While BDG detection by itself is not definitive, it can add to the diagnostic process. BDG is a cell-wall polysaccharide component found in most fungi such as *Candida* species and *Aspergillus* species. The measurement of BDG in serum is widely used as a diagnostic marker for invasive fungal infections in Japan. The clinical utility of BDG detection has also been reported outside of Japan (Odabasi et al. 2004; Ostrosky-Zeichner et al. 2005; Pickering et al. 2005).

While clinically useful, BDG testing must be utilized in conjunction with other diagnostic tests and correlated with clinical findings, because false positives were reported

in the following patients: those undergoing hemodialysis with dialysis membranes composed of cellulose (Taniguchi et al. 1990; Kato et al. 2001), receiving certain blood products such as albumin and immunoglobulins (Usami et al. 2002; Nagasawa et al. 2003; Ogawa et al. 2004), undergoing treatment with glucan-containing anti-cancer drugs such as lentinan and sizofiran (Ishizuka et al. 2004), or receiving intravenous antimicrobials (Francisco et al. 2006), and patients exposed to gauze or sponges containing BDG during surgery (Kimura et al. 1995; Nakao et al. 1997a, 1997b).

In our institution, we encountered a patient who exhibited a highly elevated BDG value of 2964 pg/ml (cut-off for a negative of 11 pg/ml) when tested on the day following a lung transplant (Oishi et al. 2006). The patient was a 37 year-old female with pulmonary-lymphangiomyomatosis who underwent lung transplantation. As a result of extended surgery length necessitated by removal of pleural adhesions, the patient lost a significant volume of blood, requir-

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ing the extensive use of gauze along with the use of an artificial heart-lung machine and a blood pump for returning blood loss to the circulation. Since this patient demonstrated no evidence of an invasive fungal infection and the fact that the serum specimen that tested positive was collected on the day following surgery, we felt that the elevated BDG value was not consistent with a fungal infection. As the patient was not exposed to cellulose membranes, certain blood products as well as drugs containing glucan during surgery, we suspected the extensive use of gauze (LAP sponges) during surgery was associated with the elevated BDG.

The mechanism by which BDG is released from gauze is not understood. There have been no previous reports to quantitatively correlate the amount of gauze usage and BDG levels. In this study, we extracted BDG from various gauze products and measured BDG to better understand the degree of which gauze contributes to elevated BDG values.

MATERIALS AND METHODS

Gauzes tested

Six types of gauze products were tested. Table 1 lists the gauze along with a description of how they are used in the clinical setting. LAP sponges (LAP sponge[®], Kawamoto Industry, Osaka, Japan and Ope-ze HP[®], Hakujuji, Tokyo, Japan), were used as bleeding during surgery was heavy. A sample of 0.5 g was cut from the LAP sponge, placed in individual sterile bags (HOGY Medical Co., Ltd, Tokyo, Japan) and sterilized in an ethylene oxide gas sterilizer.

Water used to elute β -D-glucan from gauze

Two water purification systems were used. Water was initially purified by Pure Port[®] (SHIBATA Scientific Technology, Ltd, Tokyo, Japan) and then processed by Milli-Q[®] (Japan Millipore Corporation, Tokyo, Japan). Water purified through both systems was sterilized by autoclaving at 121°C for 15 min. Purified water samples without gauze were used as negative controls (< 4 pg/ml).

Glassware and pipet tips

Erlenmeyer flasks used in the study were washed with purified water and autoclaved. Pipet tips used were certified to be glucan-free (Greiner Ultratip[®], Greiner Bio-One, Frickenhausen, Germany). Tips as well as all other equipment used in the study were sterilized by autoclaving.

Extraction and measurement of β -D-glucan from gauze

Each gauze sample was aseptically removed from its aseptic bag and immersed in an Erlenmeyer flask containing 100 ml of purified, sterile water and a magnetic stir bar. The flask was placed on a magnetic stirrer (SR-200[®], Sansyo Co., Ltd, Tokyo, Japan) set at a speed of 1000 rpm/min for 2.0 h in a 37°C incubator. The purified water was sampled at 0, 30, 60 and 120 min. Water samples were held at -80°C until testing. At each sampling interval, control purified water samples not containing gauze were handled in the same manner as purified water containing gauze.

BDG assay

The BDG assay (β -Glucan test Wako[®], Wako Pure Chemical Industries, Ltd, Osaka, Japan) was performed as specified by the manufacturer. Briefly, eluates from the gauze were added to a pre-treatment solution containing a surfactant and polymixin B and treated for 10 min at 70°C to eliminate the effect of Factor C. In the BDG assay, a trace amount of BDG triggers a horseshoe crab coagulation reaction through factor G with a dynamic range of 6 pg/ml to 600 pg/ml (<http://www.wako-chem.co.jp/rinyaku/products/biseibutu/index.html>). Elution samples exceeding 600 pg/ml were diluted in purified water and retested. Each eluate was tested three times.

Statistical analysis

Data were analyzed using GraphPad Prism[®] statistical software for windows (GraphPad Prism[®] version 4.0, GraphPad Software, Inc, San Diego, CA, USA). Differences between groups were examined for statistical significance using one-way analysis of variance (ANOVA). *P* values less than 0.05 were considered significant.

TABLE 1. Gauze tested.

Gauze	Raw Material	Fabric	Main Use
absorbent gauze JP* type I (gauze [®])	Cotton	Woven	Absorption of blood and body fluid. Mainly used in the outpatient and hospital ward setting.
nonwoven surgical dressing made of cotton (single gauze [®])	Cotton	Nonwoven	Same as above
nonwoven surgical dressing made of lyocell (soft gauze [®])	Pulp	Nonwoven	Same as above
LAP sponge with X-ray detectable thread, prewashed (LAP sponge [®])	Cotton	Woven	Absorption of blood and body fluids. Mainly used in the surgery setting. Preservation and compression of transplant organs. Compared to gauze the pre-washed product has superior absorption and cushioning properties.
LAP sponge with X-ray detectable thread, non-washed (Ope-ze XP [®])	Cotton	Woven	Same as above
gauze sponge USP** type VII gauze with X-ray detectable thread (VISTEC [™] X-Ray Detectable Sponges)	Cotton	Woven	Used mainly during surgical procedures, tissue dissection and cavity absorption

LAP sponge[®] is a product of Kawamoto Industry, Osaka, Japan. VISTEC[™] X-Ray Detectable Sponges is a product of Tyco/Healthcare, Mansfield, MA, USA. The other products are manufactured by Hakujuji, Tokyo, Japan. *JP: Japanese Pharmacopoeia **USP: United States Pharmacopoeia.

TABLE 2. Comparison of levels of (1-3)- β -D-glucan released from gauze at different times of extraction.

Gauze	0 min	30 min	60 min	120 min
absorbent gauze JP type I (gauze [®])	241.0 \pm 118.7	835.0 \pm 59.0	1061.3 \pm 126.2	1314.0 \pm 72.7 ^{d,c}
nonwoven surgical dressing made of cotton (single gauze [®])	60.7 \pm 69.4	1264.3 \pm 392.0	1420.7 \pm 331.7	2044.7 \pm 535.1 ^{c,d}
nonwoven surgical dressing made of lyocell (soft gauze [®])	4.0 \pm 0	6.3 \pm 0.6	8.7 \pm 0.6	11.7 \pm 1.5 ^{a,c}
LAP sponge with X-ray detectable thread, prewashed (LAP sponge [®])	72.7 \pm 58.4	5474.7 \pm 446.3	6301.0 \pm 538.7	6612.0 \pm 538.7 ^b
LAP sponge with X-ray detectable thread, non-washed (Ope-ze XP [®])	64.3 \pm 96.0	1127.0 \pm 46.8	1315.3 \pm 121.8	1684.0 \pm 212.3
gauze sponge USP type VII gauze with X-ray detectable thread (VISTEC [™] X-Ray Detectable Sponges)	89.3 \pm 75.4	933.0 \pm 64.0	1217.7 \pm 110.3	1595.0 \pm 216.5 ^c

(1-3)- β -D-glucan values are expressed as the mean \pm s.d. (pg/ml). Purified water samples without gauze were used as negative controls (< 4 pg/ml).

^aThe lowest BDG values after 120 minutes. ^bThe highest BDG values after 120 minutes. ^c $P < 0.05$. ^d $P = NS$. ^e $P = NS$.

RESULTS

Table 2 shows the mean BDG \pm 1 s.d. values (pg/ml) of gauze eluates tested three times. The lowest BDG value was seen in nonwoven surgical dressing made of lyocell (Soft gauze[®]) with a mean value of 11.7 pg/ml after 120 minutes of extraction in purified water. The other surgical gauzes tested showed various levels of BDG at different times of extraction. The highest BDG value was seen with prewashed LAP sponge with X-ray detectable thread (LAP sponge[®]) with a mean value of 6612 pg/ml after 120 minutes of extraction.

In comparing raw material used in the manufacturing of a particular type of gauze, of the nonwoven surgical dressings, Soft Gauze[®] (pulp) showed significantly lower BDG values than Single Gauze[®] (cotton) ($P < 0.05$). With respect to BDG values by texture, there was no difference between Gauze[®] (woven) and Single Gauze[®] (nonwoven) made from cotton ($P = NS$). In a comparison of BDG values from gauze by manufacturing source, no significant differences were observed between gauze JP type I (Gauze[®]) manufactured in Japan and gauze sponge USP type VII gauze with X-ray detectable thread (VISTEC[™] X-Ray Detectable Sponges) manufactured in the United States ($P = NS$).

DISCUSSION

Current diagnostic methods for detection of fungal infections are limited. Blood cultures are positive in only about 50% of invasive candidiasis and in less than 10% of invasive aspergillosis (Ostrosky-Zeichner et al. 2005). Although a histopathological diagnosis is conclusive, the procedure is invasive and not always feasible in critically ill patients. As a result, the measurement of BDG is a useful diagnostic marker for invasive fungal disease. The major limitations of the assay are the medical sources of BDG that

can lead to a false positive assay result.

In this study, we evaluated the β -Glucan test WAKO[®] which specifies pretreatment of serum by dilution and heating with detection by kinetic turbidimetry. Of the BDG detection methods available in the market, the β -Glucan test WAKO[®] has been reported to be highly specific, due to the assay not reacting to endotoxin. The other methods for BDG detection are pretreatment by dilution and heating with detection by an endpoint chromogenic assay and alkali pretreatment with kinetic or endpoint BDG determinations. Of the BDG assays in the marketplace, differences in sensitivity, specificity and measurement values among the commercial kits have been reported (Hossain et al. 1997; Yoshida et al. 2002; Moro et al. 2003). These differences are believed to be related to variables including differences in pretreatment, detection methodology, cut-off value, standard β -glucans and species of horseshoe crab as a source of reagent.

In testing our hypothesis that the abnormally high BDG value observed in the patient was related to the quantity and type of gauze used during surgery, we systematically compared the degree to which BDG values differ by surgical gauze brand. In recent years, gauze with different properties including increased absorbing capacity, less abrasion, decreased penetration of gauze fibers into tissue and low fuzz have been increasingly used in clinical practice. In addition, nonwoven gauze has been developed. With the exception of nonwoven surgical dressing made of lyocell (Soft Gauze[®]), there was a correlation between extraction time in purified water and an increase in BDG values. While it has been previously reported that gauze can contribute to false positive BDG values, this is the first to systematically study gauze and false positive BDG values.

The major component of gauze consists of cotton-derived material containing cellulose in the form of a linear chain several hundred to over nine thousand (1-4)- β -

D-glucan which differs from BDG found in fungal cell walls (Shepherd 1987; Beauvais et al. 2001). Hemodialysis membranes made of cellulose may contain both BDG and (1-4)- β -D-glucan. Kanda et al. (2001) found that cellulose hemodialysis membranes caused false positive BDG results but regenerated cellulose (cellulose triacetate) membranes did not. The manufacturer has claimed that BDG detection by its limulus-based assay does not react to (1-4)- β -D-glucan (http://www.wako-chem.co.jp/siyaku/info/life/article/talk_lal.htm; Tanaka et al. 1991).

We observed different BDG values based on the brand and type of gauze. Pre-washed LAP sponge with X-ray detectable thread (LAP sponge[®]) released a significantly higher level of BDG compared to the non-washed product (Ope-ze XP[®]) ($p < 0.05$). Pre-washing of cotton fibers removes natural oils and impurities thus rendering the fibers more absorbent and improving the sponge-like appearance; however, our results clearly show that the pre-washing step results in elevated BDG values compared to material that has not been pre-washed.

Based on our experimental analytical data, we are able to correlate LAP sponge usage during surgery and BDG values contributed by gauze in sera. Assuming a typical adult has a blood volume of 5000 ml, a blood loss during surgery of 1000 ml would result in the use of 300 g of LAP sponge. If one assumes that extracorporeal circulation equipment allows the return of blood loss of 100 ml to the circulation, the projected BDG value would be 7934.4 pg/ml. This value is consistent with our experimental data where extraction with purified water for 120 minutes resulted in a BDG value of 6612 pg/ml.

When gauze is used extensively during the course of surgery, it may not be possible to initially differentiate a true invasive fungal infection or an artifact resulting from gauze; there is a need to diagnose an invasive fungal infection by judging it from not only BDG values, but also clinical manifestations, antigen testing and fungal cultures. Our study has allowed us to improve our interpretation of elevated BDG values. On the other hand, in assessing the efficacy of antifungal therapy and monitoring BDG levels during the course of an invasive fungal infection, the half-life of BDG should be clarified for patient management. Kanda et al. (2001) reported that the median half life of BDG was 20 hours.

Our data shows that nonwoven surgical dressing made of lyocell (Soft Gauze[®]) did not generate elevated BDG values when extracted for 120 minutes. The reason is not clearly understood and additional studies are necessary to fully understand the manufacturing process leading to no leaching of BDG from gauze material. Lyocell is a form of regenerated cellulose in which wood pulp is dissolved in an amine oxide solvent, and cellulose fibers are then precipitated from solution. This process is similar to that used for rayon and triacetate. Hemodialysis membranes made of triacetate did not cause false positive BDG results in a previous study (Kanda et al. 2001). This suggests further studies

to test gauze or absorbent pads made of other regenerated cellulose materials. On the basis of our study, the use of nonwoven surgical dressing made of lyocell (Soft Gauze[®]) appears to be the most suitable product for use during surgery; however, it not necessarily the most ideal product in a surgical setting. More β -glucan-free gauze products are needed in the marketplace.

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