Quantification of Arabinitol in Serum by Selected Ion Monitoring as a Diagnostic Technique in Invasive Candidiasis

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D-Arabinitol was identified by mass spectrometry as a metabolite of *Candida albicans* and *Candida tropicalis*. For quantification, serum was deproteinized with acetone, the supernatant was evaporated to dryness, the silyl derivative was formed, and a portion was injected into a combined gas chromatograph-mass spectrometer system. Erythritol or 2-deoxy-galactitol was the internal standard. The protonated molecular ions, obtained in chemical ionization with isobutane, were monitored. For 39 normal subjects the mean endogenous arabinitol concentration was 0.52 $\mu\text{g/ml}$ (standard deviation, ± 0.34). An increment of 0.2 μg of arabinitol per ml could be quantified. Of 11 cases with diagnosed invasive candidiasis, 9 had arabinitol levels $>1.2 \mu\text{g/ml}$ (8 μM) in the range of 1.2 to 25.0 $\mu\text{g/ml}$; the remaining 2 cases had levels in the normal range. Six cases of diagnosed colonized candidiasis showed normal arabinitol levels.

Infection as a contributory cause of death in cancer patients may reach as high as 50% depending on the type of underlying disease (5). Fungal infection occurs at some time in 10 to 25% of all cancer patients. It has been reported that fungal septicemia in patients with acute leukemia has increased from 2 to 6% during the last 5 years (3). In a recent study of 42 autopsies performed on leukemia and lymphoma patients, severe fungal superinfections, predominantly candidiasis and aspergillosis, were found in 52%; most of the patients were not diagnosed and hence were not treated antemortem (11). Hospital-acquired fungemia also appears to be on the increase in patients with a variety of diseases and who have undergone surgical procedures, due to the use of antibiotics, indwelling catheters, and parenteral hyperalimentation (6).

Episodes of fungemia often resolve spontaneously without the need of potentially toxic antifungal therapy (2). In immunosuppressed patients, however, fungemia is usually not transient and is often invasive in nature. By invasive we mean the infiltration and colonization of the microorganisms into normally sterile deep-seated tissues and organs. Probably as many as 90% of all invasive *Candida* infections are not diagnosed early enough to permit effective treatment. Reasons for the lack of recognition include the protean nature of symptoms as well as the lack of rapid and reliable diagnostic tests. In a recent review of the available diagnostic techniques (10) the authors concluded that "traditional cultural and serological determinations of antibody response to *Candida* have not been

satisfactory for the diagnosis of invasive *Candida* infection, particularly in the host with compromised resistance." Conventional blood cultures were reported positive in 50 to 70% of cases with disseminated *Candida* infection (7). However, positive blood cultures are not always diagnostic of invasive disease because they may result from transient candidemia or from contamination of needles. Also, often the *Candida* organisms cannot be recovered (cultured) from the bloodstream of patients with deep-seated invasion.

Miller et al. (8) reported a characteristic peak in the gas chromatographic profiles of extracts from patients with candidiasis. The peak was tentatively identified as mannose, presumably originating from the mannan content of the cell wall of *Candida*. In another approach, Weiner and Yount (13) used hemagglutination inhibition to detect mannan in human serum; in sera collected serially, mannan antigenemia was detected early in the course of systemic infection in only 4 out of 14 patients.

The objective of this paper is to present methodology for the quantification of arabinitol in the serum of patients suspected of having invasive *Candida* infection. Arabinitol has been shown to be a metabolite of *Candida albicans* and *Candida tropicalis*, and elevated levels appear to be associated with invasive candidiasis. The technique described is based on mass-spectrometric monitoring of selected ions of arabinitol and an internal standard. This approach has the advantages of inherent specificity for arabinitol as well as a capability to reliably quantify

small increments in concentration. Concurrently with and independently from the present work, Kiehn et al. have developed a gas-chromatographic technique for the quantification of arabinitol in serum as a potential diagnostic tool for systemic candidiasis (4). The conclusions of our work are similar to those in their paper. The advantages of the mass-spectrometric approach are herein discussed.

MATERIALS AND METHODS

Chemicals and reagents. D-Arabinitol (arabitol), *i*-erythritol (*meso*-erythritol), D-adonitol, and D-xylitol were purchased from Sigma Chemical Co. (St. Louis, Mo.). The silylating reagent *N,O*-(bis)trimethylsilyltrifluoroacetamide + 1% trimethylchlorosilane (BSTFA + 1% TMCS), silylation grade pyridine, and acetic anhydride were purchased from Pierce Chemical Co. (Rockford, Ill.). Gas chromatographic column materials were from Supelco, Inc. (Bellefonte, Pa.). All solvents were of "distilled-in-glass" quality (Burdick & Jackson Laboratories, Muskegon, Mich.). Gas chromatographic carrier and chemical ionization reagent gases were of "high-purity" grade (Matheson Scientific, Inc., Rutherford, N.J.). Culture media and related materials were purchased from BBL Microbiology Systems (Cockeysville, Md.). Membrane filters (0.45 μ m) were from Millipore Corp. (Bedford, Mass.).

2-Deoxy-galactitol, one of the internal standards used, was synthesized from 2-deoxy-galactose (Sigma Chemical Co.) by NaBH₄ reduction, as follows. The sugar was reacted with a 4 M excess of NaBH₄ in aqueous solution for 1 h at room temperature. The reaction was stopped by the dropwise addition of acetic acid (50%) until no more hydrogen gas was evolved. The solution was evaporated with nitrogen to near dryness, and 1 volume of 20% acetic acid in methanol was added. The solution was reevaporated, and the procedure was repeated three more times to ensure the removal of excess borate. Finally, the solution was evaporated to dryness, and a stock solution (in 10% methanol-90% distilled water) was prepared. Purity was checked by forming the *O*-methyl-oxime-trimethylsilyl derivative to prove that no unreacted 2-deoxy-galactose remained and also by forming the pentaacetate derivative to prove that the total amount of sugar impurity present was <5%.

Candida cultures. *C. albicans* and *C. tropicalis* were isolated from clinical specimens submitted to the microbiology laboratory of Mount Sinai Hospital. Identification was based on sugar fermentation and assimilation studies and chlamydo-spore formation on corn-meal Tween-80 agar. Stock cultures were subcultured weekly on beef heart infusion agar and maintained at room temperature. Cultures were grown in yeast nitrogen base broth supplemented with 1% dextrose. The broth was prepared according to the manufacturer's instruction by dissolving 6.7 g of yeast nitrogen base and 10 g of dextrose in 10 ml of sterile distilled water, followed by gentle stirring and heating until dissolved. The solution was filter sterilized and stored at 4°C. Before use a portion was diluted 1:10 in sterile distilled water. A 5-ml portion of this medium

was inoculated with single-colony isolates. The cultures were grown unshaken at 37°C for 18 h. For growing *Candida* species in human serum, pooled serum was first heat inactivated at 56°C for 30 min and then inoculated and incubated as described above. After incubation, the bulk of the culture was removed by centrifuging at 1,500 $\times g$ for 10 min. For removing any residual organisms, the supernatant was filtered through a 0.45- μ m membrane filter.

For determining the number of organisms per milliliter of medium, samples from the cultures were diluted 1:10³ with normal saline, and a 0.1-ml portion was plated on 5% sheep blood agar. After incubating at 37°C for 24 h, the colonies were counted, and the number of yeast cells per milliliter of medium was calculated.

Cell hydrolysis. A 1-volume amount each (typically 0.2 ml) of fungal cells, cell-containing serum, or filtered (cell-free) serum was diluted with 1 volume of 8 N methanolic hydrochloric acid and hydrolyzed in a sealed container at 80°C for 60 min. After neutralizing with solid K₂CO₃ to pH 6 to 7, the liquid was transferred into a Corex tube with the aid of 2 volumes of methanol and centrifuged at 27,000 $\times g$ at 0°C for 15 min. The supernatant was decanted and evaporated to dryness with dry nitrogen in a water bath kept at 50°C. The dry residue was silylated as described in the section on sample preparation. The pellet remaining after centrifugation was discarded.

Computer searching for characteristic metabolites. A simple computer program was developed for the point-by-point comparison of the elution profiles obtained by collecting complete mass spectra of all chromatographically separated components from normal serum and a serum in which *Candida* was grown. The program compared each mass in the two profiles on a segment-by-segment (peak-width) basis by summing the respective ion intensities and taking their ratios. When the ratio exceeded a user selected value (threshold), the location (scan number range) and the numerical value of the ratio were printed out.

Standards and calibration samples. Both erythritol and 2-deoxy-galactitol were used as internal standards. The pure compounds were dissolved in water-methanol (9:1) to provide solutions of known concentrations. An adequate amount of internal standard was added to yield a final concentration of 1 to 2 μ g of either sugar or both sugars per ml of serum.

For establishing optimal analytical conditions both for sample preparation and also for chromatographic separation, pooled normal serum samples were spiked with known quantities of arabinitol and the internal standards. For obtaining calibration curves for quantification, samples containing increasing quantities of arabinitol and a fixed amount of internal standard were prepared. The concentration range of the calibration samples covered the entire range of concentrations expected in the samples from the patients. The amount of internal standard added was the same for both calibration samples and samples from the patients. A full set of calibration samples was analyzed with every set of samples from the patients.

Preparation of serum samples. Blood samples were drawn into a tube containing no heparin or

preservative. Serum was obtained by letting whole blood clot at room temperature for 20 to 25 min, followed by centrifugation at ca. $500 \times g$ at room temperature for 10 min. Serum samples were stored at -20°C until used.

TMS technique. The trimethylsilyl (TMS) technique was performed as follows. To a 0.2-ml sample in a glass test tube (10 by 75 mm) the internal standards were added and mixed by fast blending in a Vortex mixer for 15 s. For precipitating proteins, 1 ml of acetone was added, and the tube was centrifuged at $1,200 \times g$ for 15 min at room temperature. The supernatant was removed from the tightly packed precipitate and evaporated to dryness in a small screw-cap vial with the aid of dry nitrogen, in a water bath kept at 50°C .

The solid residue was silylated by adding 200 μl of BSTFA + 1% TMCS-pyridine mixture (3:1) and heating at 100°C in a metal heating block for 5 min. The silylation vial was sealed tightly with a Teflon-faced disk. A 4- μl portion was injected into the gas chromatograph-mass spectrometer system.

Pentaacetate technique. To a serum sample of 0.2 to 2.0 ml, 4 volumes of acetone were added, and the precipitated proteins were removed by centrifuging at $1,200 \times g$ for 15 min at room temperature. The supernatant was evaporated (dry nitrogen) to approximately 0.2 ml and diluted with 0.8 ml of distilled water. After acidifying with 2 drops of 6 N HCl, lipids were extracted twice with 3 ml of ethyl acetate, and the aqueous layer was evaporated under vacuum. The residue was acetylated with acetic anhydride-pyridine (2:1 by volume) for 2 h at 60°C in a metal heating block, the reaction was stopped by adding 1 ml of distilled water, and the acetates were extracted three times with 2 ml of hexane-chloroform (3:1 by volume). The organic extract was backwashed twice with 2 ml of distilled water, dried with solid Na_2SO_4 , and evaporated to dryness with nitrogen gas. The residue was dissolved in 100 μl of hexane, and a 4-ml portion was injected into the gas chromatograph-mass spectrometer system.

Gas chromatography-mass spectrometry. All quantification work was carried out with a combined gas chromatograph-mass spectrometer-computer system (model 3300 quadrupole-type mass analyzer and model 6000 computer; Finnigan Corp., Sunnyvale, Calif.) equipped with a chemical ionization source and computer capability for selected ion monitoring. High-precision mass measurements were made with a combined gas chromatograph-mass spectrometer-computer system (model MM 70-70 magnetic double-focusing mass analyzer and model DS 2250 data system; VG-Organic, Ltd., Altrincham, Cheshire, England).

In the TMS technique, a glass column (inside diameter, 1 m by 2 mm) filled with 3% OV-17 (80% methyl-20% phenyl silicone gum; Ohio Valley Specialty Chemical, Inc., Marietta, Ohio) on Chromosorb W HP (80/100 mesh) was employed for chromatographic separation. Depending on which internal standard was used, the column was operated isothermally at 200°C (2-deoxy-galactose) or 175°C (erythritol). The injector temperature was kept at 250°C . Trapped endogenous constituents from serum samples were removed periodically by heating the column to

250°C and keeping it at that temperature until no more effluent could be detected. The sample port was cleaned after every 20 to 25 analyses by replacing 1 to 2 cm of column material from the top of the column (dark deposits).

In the acetate technique, a glass column (inside diameter, 1.5 m by 2 mm) filled with 3% SP 2340 (75% cyanopropyl-25% methyl silicone gum; Supelco, Inc.) (100/120 mesh) was employed isothermally at 220°C ; the injector temperature was 250°C .

Isobutane was used both as the gas-chromatographic carrier gas and as the reagent gas in the chemical ionization source. There was no separator between the chromatograph and the ion source; the connecting tube was kept at 240°C . The pressure of isobutane in the ionization source was adjusted to yield the highest intensity for the $m/e = 414$ peak of perfluorotributylamine.

Operational parameters of the mass spectrometer were adjusted daily for maximum sensitivity at a resolution of about 600 with the TMS derivative of pure arabinitol. The emission current was 0.4 to 0.8 mA, and the ionization energy was 100 eV. The electron multiplier was operated with 3 kV on the conversion dynode and 1.2 kV on the amplification stage; the preamplifier was set at 10^{-8} A/V. Mass spectra for identification were obtained in the full-scanning mode. For quantification the selected ion-monitoring mode was employed. The effluent was vented for 30 s to avoid contamination of the ion source by the excess silylation reagent and pyridine.

The following masses were monitored in the TMS technique: $m/e = 513$ (arabinitol), $m/e = 411$ (erythritol), and $m/e = 527$ (2-deoxy-galactitol). Pentaacetates were analyzed by monitoring the base peak ($M - 59$) at $m/e = 303$.

The high-resolution mass-spectrometer system utilized gas-chromatographic columns and analytical conditions similar to those described above. Helium served as the chromatographic carrier gas; it was removed with a heated glass jet separator. For chemical ionization, the mass spectrometer ion source was pressurized directly with isobutane. The scan cycle was 2.4 s/decade. The ion acceleration voltage was 4 kV, and the resolution was set to ca. 10,000. Mass measurements were made both by manual peak matching and with the aid of the computer.

RESULTS AND DISCUSSION

Identification of arabinitol. Because invasive *Candida* infections are often present when the organisms cannot be recovered from blood cultures, a metabolite which could be detected in blood serum was sought as a sensitive marker that might indicate *Candida* infection. Accordingly, both *C. albicans* and *C. tropicalis* were grown in human serum for 18 h, cells were removed from the sample, and the remaining serum was filtered (0.45- μm membrane) and treated as described above. Figure 1A shows the total ion current profile (reconstructed gas-chromatographic profile) of a serum sample in which *C. albicans* was grown. These profiles are nor-

FIG. 1. (A) Reconstructed chromatogram profile (total ion monitoring) of human serum in which *C. albicans* was grown. (B) Mass chromatogram of $m/e = 513$ of A. Rel. int., Relative intensity.

malized plots of the sum of the ion abundances in each mass spectral scan as a function of the scan number; i.e., every scan represents a complete mass spectrum which is stored in memory. The profile shown in Fig. 1A could not be visually distinguished from that of a normal serum sample. However, computer comparison revealed a significant difference for mass 513 in the area of scan 30. The basis of the computer comparison was the matching of individual masses recalled from the full mass spectra (known as mass chromatograms). The peak around scan 30 in the mass chromatogram for mass 513 (Fig. 1B) of the infected serum sample was several hundred times larger than that of the control serum. The magnitude of this component appeared dependent upon the number of *Candida* organisms present.

When the complete mass spectrum corresponding to scan 30 was recalled (Fig. 2A), the abundance of mass 513 was the highest in the spectrum (base peak). This mass spectrum was identical to that of pure (silylated) arabinitol (Fig. 2B); here mass ($m/e = \text{mass}/\text{charge ratio}$) is plotted against normalized abundance. The base peak corresponds to the protonated molecular ion of arabinitol, with the uptake of five TMS groups by the molecule. The small peak at

$m/e = 423$ corresponds to the loss of an *O*-trimethylsilyl group.

Figure 3 shows a total ion current profile of hydrolyzed and silylated *C. albicans* cells. Approximately 10^7 cells were grown, the cells were removed by centrifugation, and the cell pellet was hydrolyzed and subsequently silylated. When scan 63 was recalled from the computer memory and corrected for background, the mass spectrum was identical to that of pure arabinitol (Fig. 2). The other peaks in Fig. 3 were not identified because they either did not appear in the serum after the organisms were removed or could not be separated from interfering endogenous serum constituents.

Because the $m/e = 513$ peak was also detected in all control samples, identification by high-precision mass measurement was warranted. The peak assumed to be endogenous arabinitol gave a precise mass of 513.272, corresponding to the molecular composition of $C_{20}H_{53}O_5Si_5$, which is the formula of the protonated silyl derivative of arabinitol. The mass measurement (average of seven scans) was within 1.9 millimass units of the mass calculated from the formula. The same mass and formula were obtained for the compound originating from *Candida* grown in serum as well as for the one obtained directly from the microorganism upon hydrolysis. Arabinitol was found to be a metabolite of both *C. albicans* and *C. tropicalis*.

Figure 4 shows the monitoring of arabinitol levels in two samples from a patient with acute myelocytic leukemia. Here only the protonated molecular ions of arabinitol and erythritol were monitored to achieve maximum sensitivity (while retaining specificity). The sample shown in Fig. 4A exhibits a normal endogenous arabinitol level, corresponding to $0.7 \mu\text{g}/\text{ml}$. Figure 4B shows an increase in the level to $2.5 \mu\text{g}/\text{ml}$, coinciding with the diagnosis of invasive candidiasis based upon positive culture from a liver biopsy specimen.

Because the TMS technique does not fully resolve arabinitol and the two other pentitols, adonitol (ribitol) and xylitol, a technique based on the pentaacetate derivatives was also developed. The chemical ionization (isobutane) mass spectrum of the pentaacetate of arabinitol (Fig. 5) yielded a detectable but weak $(M + 1)^+$ ion. The base peak was the $(M - 59)^+$ ion at $m/e = 303$, corresponding to the loss of a CH_3COO group. Figure 6A shows chromatographic separation for equal amounts of the three pentitols by monitoring the $m/e = 303$ ions. Figure 6B shows the monitoring of pentitols in the serum of a patient with disseminated *C. albicans* infection. The levels of adonitol and xylitol were $<10\%$ that of arabinitol in the majority of normal

FIG. 2. (A) Mass spectrum (isobutane) of scan 30. (B) Mass spectrum (chemical ionization with isobutane) of the TMS derivative of authentic arabinitol. Rel. int., Relative intensity.

FIG. 3. Reconstructed chromatogram profile of hydrolyzed *C. albicans*. Rel. int., Relative intensity.

samples as well as in those from infected patients. On a few occasions the level of adonitol was as high as 50% that of arabinitol. We have not found any evidence that adonitol or xylitol was a metabolite of *Candida* species grown in human serum.

The TMS technique is considerably faster (12 patient samples together with 6 calibration samples can be analyzed by a technician in 7 h),

simpler, and more sensitive than the one based on the separation of pentaacetates. All levels quoted for arabinitol by the TMS technique might include contributions from endogenous adonitol or xylitol or both. In case of doubt or when additional confirmation is warranted, the relative quantities of all three pentitols may be determined with the pentaacetate technique.

The possibility that arabinitol might also be

FIG. 4. (A) Single ion monitoring of $m/e = 513$ (arabinitol) and $m/e = 411$ (erythritol, internal standard [int. std.]) in a leukemic patient without infection (normal range). (B) Data for the same patient as in A at the time of diagnosed (liver biopsy) invasive candidiasis.

FIG. 5. Mass spectrum (chemical ionization with isobutane) of the pentaacetate derivative of authentic arabinitol. Rel. int., Relative intensity.

FIG. 6. (A) Single-ion monitoring of $m/e = 303$ ($M - 59$ peak) of the pentaacetates of adonitol, arabinitol, and xylitol in a calibration mixture. (B) Single-ion monitoring of the pentaacetates in the serum of a patient with invasive candidiasis.

a metabolite of other microorganisms which frequently infect immunosuppressed patients was also investigated with the computer program to compare profiles of normal serum and of serum samples in which *Aspergillus fumigatus*, *Aspergillus niger*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* were grown. No detectable differences were found between normal and infected serum samples with any of these organisms. Other relevant organisms (e.g., *Cryptococcus*, *Histoplasma*) are being investigated.

Speculations about the biochemistry of the formation of arabinitol as a metabolite of *Candida* are beyond the scope of the present work. Based upon studies on the utilization of sugars by yeasts (1), a possible route of the catabolism of L-arabinose to L-arabinitol by the *Candida* organism may take place with the aid of nicotinamide adenine dinucleotide phosphate and a dehydrogenase (12). Arabinose was not present in the serum in which *Candida* species were grown. It is presumed that the organism makes and excretes arabinitol from unknown precursors. Another, though less likely, explanation for the presence of arabinitol may be that arabinitol from dead and decomposing cells of *Candida* is continuously washed out into the bloodstream from the site of the primary infection.

Quantification. Erythritol and 2-deoxy-galactitol were selected as internal standards because their extraction, chromatographic, and mass spectral properties closely parallel those of arabinitol. The mass spectra of the silylated internal standards exhibited base peaks at $m/e = 411$ and $m/e = 527$, respectively, corresponding to the protonated molecular ions. Either internal standard was adequate; in more recent work 2-deoxy-galactitol was preferentially employed because of more convenient chromatographic behavior. In agreement with an earlier report (9), relatively large amounts of erythritol have been found in patients with renal failure. 2-Deoxy-galactitol levels were not elevated in patients with renal failure; however, other interference peaks of high abundance did appear. When interferences with the internal standard were severe, quantification of arabinitol was made with the technique of external calibration (i.e., determining arabinitol levels in the samples from patients by comparing measured areas to those obtained upon separately injecting known quantities of pure derivatized arabinitol).

A calibration curve is shown in Fig. 7. This was obtained by adding increasing amounts of arabinitol to a serum sample while keeping the concentration of the internal standard fixed. Every point on this curve corresponds to the aver-

age of four independent samples. The coefficients of variation for all points were in the range of 3.9 to 9.4%. Other reproducibility sets yielded comparable ranges. Regression analysis gave a correlation coefficient of 0.998.

Extrapolation of the calibration curve provides a value for the endogenous arabinitol in the sample when normal serum is used to establish the calibration curve. When an adequate amount of a serum sample from a patient is available, it is advantageous to use that sample as the medium to which known amounts of arabinitol are added (standard addition technique). This approach, although obviously entailing more work, provides more reliable data for patients with serum having interfering substances due to renal failure or some other cause.

When large increases occur in the arabinitol level above the level of endogenous arabinitol, gas chromatography is adequate. However, large changes are likely to be associated only with very advanced disease. For providing as early an indication of infection as possible, small incremental changes must be determined. In conventional gas chromatography identification is based only on retention time measurements. It is also difficult to quantify increments of less than a factor of two among the ever present "chemical-noise" peaks. In a series of runs with serum spiked with arabinitol in increments of 0.2 $\mu\text{g/ml}$ in the range of 0 to 1.0 $\mu\text{g/ml}$ (with four replicates at each point) we determined that an increment of 0.2 $\mu\text{g/ml}$ (1.35 μM) can be detected with selected ion monitoring. Because only preselected peaks are monitored, specificity for arabinitol is retained, and, at the same time, the presence of chemical-noise peaks is irrelevant. As shown in Fig. 4, the peak corresponding to the protonated molecular ion of arabinitol (at $m/e = 513$) is easily seen, and its area can be measured by the computer without interference or contribution from chemical noise that may occur at the same chromatographic retention time as arabinitol.

Clinical applications. Normal endogenous arabinitol levels were determined for 39 subjects (ages 16 to 59). The mean arabinitol level was 0.52 $\mu\text{g/ml}$ (standard deviation, ± 0.34). When plotted, the curve approximated normal distribution. On this basis it was estimated that there is only a 2.6% probability that an arabinitol level of 1.2 $\mu\text{g/ml}$ (8 μM) is normal.

Of 11 cases with diagnosed (autopsy or positive blood culture) invasive candidiasis (*C. albicans*), 9 had arabinitol levels $\geq 1.2 \mu\text{g/ml}$ (range 1.2 to 25.0 $\mu\text{g/ml}$); the remaining 2 cases had levels in the normal range. Six cases of oropharyngeal or vaginal candidiasis without

FIG. 7. Calibration curve for the quantification of arabinitol; normal pooled serum or the patient's own serum is spiked. Extrapolation provides endogenous (Endog.) arabinitol. For quantification of other samples endogenous arabinitol was subtracted so that the curve passed through the origin. Int. std., internal standard.

clinical findings indicative of invasion revealed arabinitol levels within the normal range.

The arabinitol levels of one patient with acute myelocytic leukemia were monitored for a period of 1 year. Arabinitol levels increased from normal to 2.5 µg/ml. Maximum levels coincided with the diagnosis of candidiasis based on liver biopsy cultures. After the initiation of antifungal therapy, arabinitol levels remained relatively high for several weeks, followed by a slow decrease to normal.

We have found that severe (requiring dialysis) kidney dysfunction can result in elevated serum arabinitol levels, leading to a false diagnosis of *Candida* infection. This was observed with patients having cancer, as well as with patients having other underlying diseases. Such cases could often be detected by strong interferences in the monitoring of the internal standard (2-deoxy-galactitol). We are currently investigating the relationship between deteriorating kidney function and serum arabinitol levels to determine how serum creatinine levels might be utilized to differentially diagnose *Candida* infection in patients with kidney dysfunction.

Early diagnosis of invasive candidiasis is essential for effective treatment. The selected ion monitoring technique described here permits the monitoring of blood levels for precise measurement of small incremental changes in arabinitol content which might provide a biochemical diagnosis of otherwise undiagnosable invasive candidal disease. The technique is sufficiently specific and sensitive to justify serial sampling in patients at high risk in an attempt to diagnose invasive candidiasis before clinical signs appear. Our data do not yet allow more than a gross

correlation between arabinitol concentration and the extent, location, or intensity of *Candida* infection. Similarly, prognostic connotations of arabinitol concentration with respect to spontaneous cure, therapeutic response to antifungal agents, and survival have not yet been established. Studies of this nature are currently in progress.

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