Biomarkers of small intestinal mucosal damage induced by chemotherapy: an emerging role for the ^{13}C sucrose breath test

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Gastrointestinal mucosal toxicity is extremely common following cytotoxic therapies. The alimentary mucosa is particularly susceptible to injury and dysfunction, leading to many debilitating complications. Despite much research, there is currently no single noninvasive biomarker to detect gut injury. Several biomarkers have been investigated in the context of gastrointestinal diseases, which may prove useful in the oncology arena. Identification of a biomarker that is easy to obtain and measure and that accurately identifies mucosal damage would allow for improved patient diagnosis of toxicities and for personalized treatment regimens. In this review, we highlight the effectiveness of urine and breath tests as potential clinically effective biomarkers, with significant focus placed on the emerging role of the carbon-13 sucrose breath test (^{13}C SBT). The ^{13}C SBT provides a simple, noninvasive, and integrated measure of gut function. The ^{13}C SBT also has the potential to monitor gut function in the setting of cytotoxic therapy–induced mucositis, or in the assessment of the efficacy of antimucositis agents.

Mucositis is a frequent, debilitating, and dose-limiting side effect of anticancer cytotoxic therapies[1] and is responsible for adverse clinical outcomes such as increased need for total parenteral nutrition; use of antibiotic therapies; risk of infection; hospitalization; and even occasionally death.[2] These clinical factors require greater resource utilization, which results in significant economic burden associated with mucositis.[2] Recent studies have identified potential biomarkers of mucositis with varying results.[3,4] This critical review will examine the noninvasive breath and urine tests as potential biomarkers for cytotoxic therapy–induced mucositis.

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Mucositis is a common but insufficiently studied complication associated with cytotoxic therapy.[2] It affects nearly all patients receiving hematopoietic stem cell transplant, and a considerable proportion of those receiving high-dose chemotherapy or radiotherapy treatment regimens.[1,4,5] Infants are at a higher risk of developing chemotherapy-induced mucosal toxicities.[6] The reduction of cytotoxic therapy–induced mucositis has been recognized as an important target for improving anticancer therapies, as well as for reducing the economic burden associated with anticancer treatments.[7] An increase in the use of multiple treatment modalities is common with advances in curative cancer treatment regimens.[1,5,7] Although this yields superior anticancer outcomes, it results in greater incidence of mucositis, highlighting the need for antimucositis-based therapies.[1] The development of effective preventive therapies has been hampered by a lack of understanding concerning the pathobiological mechanisms underlying cytotoxic therapy–induced mucositis. A hindrance in the use of mucositis biomarkers is the huge number of validated scoring systems available. These scoring systems are based on a combination of symptoms, tissue appearances, and functional changes.[8-10] However, with such a range of subjective assessment

systems, correlation with objective biomarkers may prove difficult. The difficulty in obtaining intestinal biopsy specimens to use as a clinical standard reference also presents a challenge.

The recent recognition of the complex pathogenesis of mucositis[11-13] highlights the dynamic biochemical interactions concerning the cellular constituents of the mucosa and chemotherapy agents. Chemotherapy- and radiation-induced mucositis occurs as a result of the nonselective nature of these cytotoxic agents, which are unable to distinguish between the rapidly replicating cells of neoplastic lesions and the highly regenerative cells of the alimentary epithelium.[14] It was originally thought that the clonogenic cell death of the basal layer was the direct effect of cytotoxic therapy on the epithelium; it is now understood that clonogenic cell death of the basal layer is insufficient to account for the extent of mucositis that is observed in cancer patients.[1] Subsequently, mucositis was defined as the collective consequence of the direct and indirect inhibitory effects of cytotoxic therapy on DNA replication and mucosal cell proliferation.[6]

The clinical symptoms of mucositis are not directly life threatening in most cases. The introduction of multiple treatment modalities has resulted in more severe mucositis, with alimentary tract (AT) damage becoming more frequent.[1,15] Patients with intestinal mucositis may experience symptoms including—but not limited to—nausea, vomiting, abdominal bloating, cramping, diarrhea, and rectal bleeding.[1,7,16] In more severe cases, mucositis can result in bacterial translocation and sepsis.[4] Subsequent dose limitations, parenteral nutrition, and extended hospital stay yield a reduced quality of life for the patient and an increased economic burden.[2] The gastrointestinal tract (GIT) distal to the oral cavity is largely inaccessible, making it difficult to assess. This highlights the need for an effective, noninvasive biomarker to detect GIT damage, enabling a tailored anticancer treatment regimen to be developed.

According to the National Cancer Institute, a biomarker can be defined as "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition".[17] In the search for a gastrointestinal toxicity biomarker, investigators have proposed that the biomarker be easy to access or administer by the clinician, and be able to act independently of both the patient's medication and his or her individual lifestyle requirements.[18] Thus, according to these suggestions, the most likely source of gastrointestinal biomarkers would be in the patient's blood, saliva, or feces, as these can be easily collected in quick succession on multiple occasions.[3] However, expired "breath" and the so-called "breath tests" may be the silver bullet in the search for biomarkers of gastrointestinal toxicity. This review will critically analyze urine and breath tests as potential clinically effective biomarkers in terms of their sensitivity, applicability, cost, and ability to preserve patient comfort and health, thus reducing hospital stays. Significant focus will be placed on the emerging role of the carbon-13 sucrose breath test (^{13}C SBT) as the biomarker with the greatest potential for clinical use. The metabolic background and characteristics of the ^{13}C SBT will be discussed in detail, with an emphasis on its superiority in terms of clinical application and mucositis monitoring in patients undergoing cytotoxic therapy.

Candidate biomarkers of mucosal damage

Mucositis has been substantially assessed in the literature; however, methodology and results are confined predominantly to oral mucositis,[2,19-21] primarily because of the relative accessibility of the oral cavity. The inaccessibility of the gut distal to the oral cavity has rendered that region of the AT difficult to objectively assess in its entirety. In addition to the isolation of the intestine, the pathogenesis of intestinal mucositis differs significantly from that of oral mucositis in terms of the onset of pain and ulceration.[15] The onset of oral mucositis is not an effective predictor of intestinal mucositis.[1,5]

Conventional techniques that determine GIT function—such as the very early Crosby capsule, endoscopy, sigmoidoscopy, and colonoscopy—were advances into monitoring the health of the bowel.[4,22] Although endoscopy and colonoscopy are used widely in clinical settings to aid the diagnosis of AT conditions, they lack the ability to assess the intestine in its entirety, as only the proximal portions of each region can be routinely assessed.[4] These techniques have taken time to become established within oncology treatment settings because of their perceived risks with respect to invasive procedures during postchemotherapy neutropenia and thrombocytopenia.[23] In spite of evidence indicating that these conventional procedures are safe and effective in postchemotherapy patients,[23,24] the current clinical standard technique for assessing the integrity of the intestine remains the bowel biopsy.[4] Biopsies, however, have a number of inadequacies regarding assessment of bowel function; namely, the procedure is confined to the proximal portions, is painful and expensive, requires sedation, and provides an indication of health only in the portion of the bowel that is biopsied.[25]

Although cytotoxic therapy–induced mucositis is unlikely to be expressed or quantified by a single parameter, a single biomarker for intestinal mucositis would be highly beneficial. Furthermore, biomarkers that not only

determine the presence of mucositis but also act as a predictor of injury or damage resolution would be highly valuable clinically. Such a biomarker would allow for improved patient monitoring; the formation of a tailored, personalized, treatment regimen; and possible early release from the hospital.[3] Ideally, such an assay would be tissue specific, would display a dose-response relationship, would be easily accessible in clinical practice, and would be independent of experimental conditions, medication, and nutritional status.[7,26] In addition, the marker would preferably be noninvasive in order to preserve patient comfort and reduce hospital stays and economic impact.[7]

Sugar permeability test

The permeability of the small intestine has been used to indicate barrier function in a relatively noninvasive fashion. In a healthy GIT, disaccharides do not cross the mucosa to any appreciable extent.[27] Methods utilizing differential absorption of monosaccharide and disaccharide in the small intestine, such as L-rhamnose and lactulose, were first implemented to assess sugar permeability in celiac patients with villous atrophy.[28] The sugar permeability test measures urinary excretion of orally administered nonmetabolizable sugars, and as such assesses the function of the small intestine.[23,29] The dual-permeability test using L-rhamnose and lactulose determines small bowel surface area and enterocyte tight junction concentration, respectively.[29] Several studies have shown relative success in correlating high-dose chemotherapy effects (enteropathy and permeability of tight junctions) and altered monosaccharide and disaccharide urine concentrations.[29-31] All the studies we reviewed that assessed the efficacy of the sugar permeability tests produced positive results, highlighting the modified permeability of the proximal small bowel in response to chemotherapy-induced mucositis. However, the drawbacks of the sugar permeability test include the need to collect urine over an extended period of time, as well as a requirement for highly-specialized equipment to analyze results.[29]

More recently, sucrose has been used to detect small intestine permeability. Sucrose is a disaccharide molecule composed of fructose and glucose. As a result of the actions of sucrase, a brush border enzyme, the sucrose molecule is destroyed, enabling digestion of its monosaccharide derivatives. The absorption of intact sucrose implies damage to the epithelium of the proximal small bowel;[27] thus, increased sucrose permeability should, theoretically, reflect the presence of AT damage to the proximal small bowel. Sutherland and colleagues showed that gastric ulcers and severe gastritis were detected by sucrose permeability, as assessed by urine sample.[27] The sucrose permeability test does not provide an alternative to techniques such as endoscopy; however, it may present a clinically useful technique to identify patients who would benefit from endoscopy, and thus may facilitate early detection of mucosal damage. Although the sucrose permeability test is useful in the assessment of barrier function, it lacks the ability to provide a sensitive indication of the absorptive capacity of the small intestine, as it was unable to reliably detect mild mucosal damage.[27]

For the sugar permeability test to be considered efficacious, not only does the insensitivity of the test need to be addressed, but also research must be extended to chemotherapy-induced mucosal damage and not be confined to gastritis and other inflammatory GIT diseases. Additionally, although a urine sample may be an advance on the repeated blood tests needed to assess plasma citrulline,[32-33] 5-hour urine collection from cancer patients is tiresome and often problematic.[4] These reasons reinforce the need for a simple, reliable, and noninvasive assay for the detection of intestinal mucosal damage.

Hydrogen breath test

The hydrogen breath (H_2) test is based on the principle that an unhealthy or damaged small bowel will malabsorb ingested sugar substrates. This technique allows an impaired small bowel (or one lacking specific transporters) to be detected by the increased concentration of hydrogen expelled in the breath, a consequence of the increased luminal substrate of the malabsorbed sugar component.[4] After the intact macronutrient passes through the small intestine, it reaches the colon where the substrate is metabolized by hydrogen-producing bacteria.[34] Hydrogen then enters the venous circulation and is transported to the lungs, allowing it to be expired.

Although this clinically utilized and harmless test is an improvement on alternative invasive tests, it is hampered by its inability to provide a simple measure of intestinal damage, as it provides a gauge only of the absorptive capabilities of the small intestine. Additionally, the H_2 test relies heavily on the existence of a family of hydrogen-producing bacteria of the large bowel; these bacteria are absent in approximately 20% of the population.[34-36] Cancer patients are often prescribed antibiotics that play a role in the falling levels of hydrogen-producing bacteria as part of a colonic microbiota.[34,35,37,38] A final inadequacy of the H_2 test is the subjectivity and variability in gut flora, which weakens its reputation as a potentially effective biomarker for chemotherapy-induced mucosal damage. Tooley and colleagues argued that changes in diet can significantly alter the integrity and profile of gut flora.[4] As a result, the test may yield potential false-negative results. It has been

suggested that a negative H_2 test may result from shifts in bacteria profiles and types (eg, methanogen-producing bacteria).[4]

^{13}C sucrose breath test

Koetse and colleagues concluded that using the combined ^{13}C-lactose $^{13}CO_2/H_2$ test provided superior results compared with the H_2 test alone in determining gastrointestinal damage.[34] It was also determined that the use of the ^{13}C lactose test alone lacked the capabilities to be a suitable marker of epithelial damage in the small bowel, as 80% of nonwhites showed lactase inadequacies in relation to age.[34,39] On the other hand, sucrase is a relatively stable enzyme with similar levels throughout an individual's life,[40] and only 0.2% of the human population have sucrase deficiencies,[39,40] suggesting that a test utilizing ^{13}C sucrose would prove to be reliable in the detection of mucosal damage of the small bowel.

The ^{13}C SBT is based on the principles that form the basis of the sugar permeability tests. The ^{13}C SBT relies on the detection of $^{13}CO_2$ in the breath, following an oral dose of a suitable sugar substrate.[16] In a healthy GIT, this substrate (sucrose) is cleaved into its monosaccharide constituents via the actions of sucrase, a brush border enzyme of the small intestine.[6,16] The products are metabolized by the liver and expired in the breath. Mucositis involving the small bowel is known to cause villous atrophy and reduced surface area of the GIT,[6] which consequently yields diminished sucrase activity. Therefore, it can be deduced that reductions in $^{13}CO_2$ in expired breath reflect reductions in sucrase activity, and hence reflect the extent of intestinal damage.

The development of the ^{13}C SBT occurred initially in animal models of mucositis, primarily to validate the biomarker, and then to test the efficacy of antimucositis agents.[4,16,29] This second outcome is important for the translation of agents into the clinical arena. This novel breath test was first implemented in 2004 by Pelton and colleagues to assess small intestinal sucrase activity in male Sprague Dawley rats.[41] In this study, mucosal damage was instigated via 3 daily subcutaneous injections of methotrexate (MTX), an active chemotherapy agent that disrupts normal DNA synthesis via the inhibition of specific enzymes (eg, dihyfrofolate reductase) that are necessary for maintaining GIT integrity.[40] The MTX-treated rats showed significant impairment in $^{13}CO_2$ exhalation, indicating diminished sucrase activity in the small bowel, 7 days after the treatment.[41] The study reported significant correlations ($R^2 = 0.92$) with histologic and biochemical (in vitro) measurements of GIT damage and sucrase activity, respectively.[41] This objectively indicates a strong correlation between levels of expired $^{13}CO_2$ and mucosal damage of the small intestine induced by MTX.

Another study by the same group demonstrated that calcium folinate ingestion was an effective preventative or limiting factor in mucositis development. The ^{13}C SBT was successfully applied, following MTX-induced mucositis in the rat.[42] Strong correlations ($R^2 = 0.89$) were evident between expired $^{13}CO_2$ and the levels of mucosal damage and sucrase activity, as defined histologically and biochemically. More importantly, in terms of clinical application, the ingestion of calcium folinate prior to MTX administration completely prevented the onset of MTX-induced intestinal mucositis.[42] Therefore, this study demonstrated that the ^{13}C SBT not only is a technique that monitors mucosal damage during cytotoxic therapy, but also has further applications in the screening of potential preventive agents that are targeted at minimizing the adverse effects of chemotherapy, such as mucositis.[42]

Although the previous 2 studies revealed significant findings addressing chemotherapy-induced mucositis, they were limited to just the single chemotherapy agent, MTX.[6,29] More recently, the ^{13}C SBT was successfully applied by Howarth and colleagues to reflect mucosal injury induced by a variety of chemotherapy agents.[16] Following a methodology similar to that of previous studies,[41,42] the investigators evaluated the ^{13}C SBT as an indicator for small bowel injury and dysfunction, utilizing a rat model of chemotherapy-induced mucositis. The chemotherapeutic agents utilized to induce mucosa damage included MTX, 5-fluorouracil (5-FU), cyclophosphamide, etoposide, doxorubicin, irinotecan, and a combined therapy to reflect combination therapies employed in modern anticancer regimens.[16] The ^{13}C SBT results reflected the time course of damage and repair. The investigators found a high degree of accordance between ^{13}C SBT results and the more invasive biochemical and histologic measures of GIT damage ($R^2 = 0.82$). The use of a variety of chemotherapy agents is what set this study apart from past studies, and supported the application of the ^{13}C SBT to a number of chemotherapy agents, not only antimetabolites (eg, MTX). More importantly, the use of different classes of chemotherapy agents allowed the sensitivity of the ^{13}C SBT to be addressed, as different classes of chemotherapy agents induced varied degrees of mucosal damage.[16] In this study, for example, irinotecan induced lower than expected damage to the mucosa of the small intestine. The more invasive histologic and biochemical tests were able to detect these changes, and—impressively–these alterations were reflected by the ^{13}C SBT. This indicates that the ^{13}C SBT is capable of detecting milder forms of intestinal damage, demonstrating

TABLE Strengths and weaknesses of key noninvasive tests for the detection of cytotoxic therapy–induced gut toxicity (mucositis)

	Strengths	Weaknesses
Sugar Permeability Test	• Noninvasive. • Assesses barrier function of the small intestine. • Facilitates early detection of mucosal damage. • Clinically useful in identification of patients who would benefit from further investigation (eg endoscopy).	• No alternative to invasive techniques. • Indirect measure of small intestine damage; does not clearly describe functionality of small intestine. • Tiresome, problematic regular urine collection. • Relatively nonspecific.
Hydrogen Breath Test	• Noninvasive. • Assesses small bowel function. • Clinically applied. • Minimal patient risk.	• Reflects only absorptive capacity of the small bowel. • Relies on the existence of hydrogen-producing bacteria of the large bowel (absent in 20% of the population). • Risk of false negatives from antibiotic interference or variability of the gut microflora.
^{13}C Sucrose Breath Test	• Simple breath test. • Cost effective. • Based on sucrase, a highly stable enzyme present in 99.8% of population. • Assesses both function/integrity and absorptive capacity of small intestine. • Detects time course of both damage and repair. • Detects mild forms of toxicity (sensitivity). • Successful in vivo application with various chemotherapeutic agents. • Clinical application. • Monitoring and screening potential.	• Clinical assessment limited to certain populations. • Frequency of breath tests may be problematic for patients. • Highly specialized equipment required. • Lacks investigation for radiotherapy- and myeloablative therapy–induced gut toxicity. • Relies on hepatic and respiratory function (hepatic function often compromised following cytotoxic therapies).

its superiority as a potential biomarker for cytotoxic therapy–induced mucosal damage.

Recently, Tooley and colleagues conducted the noninvasive ^{13}C SBT in rates at 3 time points—prior to tumor inoculation, prior to MTX administration and prior to sacrifice—to monitor gut function in the setting of cytotoxic-therapy–induced intestinal mucositis.[43] In addition to significantly decreased body weight, sucrase and myeloperoxidase activity, and tumor progression, the results indicated that MTX-treated rats also had significantly lower SBT levels.

A subsequent study conducted by Yazbeck and colleagues assessed the applicability of the SBT to monitor gut function in response to an antimucositis agent (palifermin).[44] Results showed reduced ^{13}C SBT values in 5-FU–treated rats, compared with untreated controls ($P < .05$), which suggested that the ^{13}C SBT can monitor the function of the gut in the setting of cytotoxic therapy–induced mucositis. These results also highlighted the ability of the ^{13}C SBT to monitor the ability of antimucositis agents to modify the functional capacity of the intestine in rats with intestinal mucositis.

The ^{13}C SBT has recently been applied to pediatric cancer patients in a clinical setting.[45] Although this study was somewhat limited by a relatively small sample size containing only children, it was able to verify the successful application of the ^{13}C SBT, with findings indicating that for the first time, it was possible to noninvasively detect and monitor chemotherapy-induced mucosal injury. In addition, the investigation highlighted the sensitivity of the ^{13}C SBT, with the test's detection of the onset of intestinal mucositis before clinically observable disease was present.[45] Larger randomized clinical studies are now warranted in both the pediatric and adult cancer setting to confirm these encouraging findings.

The successful clinical application of the ^{13}C SBT suggests that it has significant potential as a noninvasive biomarker to detect and monitor mucosal damage of the AT in cancer patients undergoing chemotherapy regimens (Table). This test is cost effective, easy to administer, and well-tolerated by patients. However, although the ^{13}C SBT has shown promise as a potential biomarker, it does have some limitations that need to be addressed in future studies. One of the major drawbacks is the time frame for the test, with patients required to have their breath collected every 15 minutes for 2 hours on multiple testing occasions. Secondly, highly specialized equipment (an isotope ratio mass spectrometer) is required for analysis of the samples (Table).[41,42,45]

A further significant limitation is that the underlying mechanism by which the ^{13}C SBT detects chemotherapy-induced mucositis relies heavily on both respiratory and

hepatic function. It has been well documented that cytotoxic therapies are associated with a high prevalence of heptaxocity including chemotherapy-associated liver injury resulting in adverse outcomes including toxic hepatitis,[46] hepatic steatosis and steato-hepatitis;[47] (McBride and colleagues, unpublished data, 2012); therefore, an assessment of liver function would be required for the ^{13}C SBT to be applied universally. However, there currently are no preventive or treatment options for chemotherapy-associated hepatotoxicity, and little research exists on the underlying pathobiological mechanisms.[48] Likewise, because respiratory function is highly variable within patients, both hepatic and respiratory dysfunction may play a role in the applicability of the test.[48]

These caveats need to be addressed before the ^{13}C SBT could be fully implemented as an effective clinically relevant biomarker (Table). In addition, to be widely accepted into the oncologic arena, it requires further preclinical and clinical research in different study populations including adults, in hematologic cancers, and in radiotherapy-induced gut toxicity.

Conclusion

This review has provided a critical analysis of breath and urine biomarkers for cytotoxic therapy–induced mucosal damage. Although it is unlikely that such damage is to be expressed by a single parameter, the ^{13}C SBT is emerging as the most effective quantitative marker of mucosal damage. The ^{13}C SBT has been successfully applied to a variety of experimental and clinical settings, highlighting its sensitivity and ability to detect and monitor small bowel function as affected by different classes of chemotherapy agents.[41,44,45] The ^{13}C SBT provides an easy and cost-effective means of detecting mucosal damage, as well as providing an early indication of intestinal injury.[45] The ^{13}C SBT has proved to be a suitable technique for the assessment of the stages of mucosal damage and its severity, perhaps allowing for therapeutic intervention to form more effective chemotherapy regimens. Additionally, the ^{13}C SBT has been used to monitor small bowel function in the presence of a range of developing antimucositis agents such as calcium folinate. For these reasons, we propose that the ^{13}C SBT is a promising candidate biomarker of small bowel function, and can be applied in clinical practice for the examination of mucositis that develops in patients who are undergoing cytotoxic therapy.

References

1. Sonis ST, Elting LS, Keefe D, et al. Perspectives on cancer therapy–induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer*. 2004;100(9 suppl): 1995-2025.
2. Elting LS, Cooksley C, Chambers M, Cantor SB, Manzullo E, Rubenstein EB. The burdens of cancer therapy. Clinical and economic outcomes of chemotherapy-induced mucositis. *Cancer*. 2003; 98(7):1531-1539.
3. Gibson RJ, Bowen JM. Biomakers of regimen-related mucosal injury. *Cancer Treat Rev*. 2011;37(6):487-493.
4. Tooley KL, Howarth GS, Butler RN. Mucositis and non-invasive markers of small intestinal function. *Cancer Biol Ther*. 2009;8(9): 753-758.
5. Keefe DM, Schubert MM, Elting LS, et al. Updated clinical practice guidelines for the prevention and treatment of mucositis. *Cancer*. 2007;109(5):820-831.
6. Sonis ST, Eilers JP, Epstein JB, et al. Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. *Cancer*. 1999;85(10):2103-2113.
7. Lutgens L, Lambin P. Biomarkers for radiation-induced small bowel epithelial damage: an emerging role for plasma citrulline. *World J Gastroenterol*. 2007;13(22):3033-3042.
8. Peterson DE, Cariello A. Mucosal damage: a major risk factor for severe complications after cytotoxic therapy. *Semin Oncol*. 2004;31(3 suppl 8):35-44.
9. Scarantino C, LeVeque F, Swann RS, et al. Effect of pilocarpine during radiation therapy: results of RTOG 97-09, a phase III randomized study in head and neck cancer patients. *J Support Oncol*. 2006;4(5):252-258.
10. Sung L, Tomlinson GA, Greenberg ML, et al. Validation of the oral mucositis assessment scale in pediatric cancer. *Pediatr Blood Cancer*. 2007;49(2):149-153.
11. Sonis ST. Oral mucositis in cancer therapy. *J Support Oncol*. 2004; 2(6 suppl 3):3-8.
12. Sonis ST. A biological approach to mucositis. *J Support Oncol*. 2004;2(1):21-32; discussion 35-36.
13. Sonis ST. The pathobiology of mucositis. *Nat Rev Cancer*. 2004; 4(4):277-284.
14. Rubenstein EB, Peterson DE, Schubert M, et al. Clincal practice guidelines for the prevention and treatment of cancer therapy–induced oral and gastrointestinal mucositis. *Cancer*. 2004;100(9 suppl):2026-2046.
15. Keefe DM. Gastrointestinal mucositis: a new biological model. *Support Care Cancer*. 2004;12(1):6-9.
16. Howarth GS, Tooley KL, Davidson GP, Butler RN. A non-invasive method for detection of intestinal mucositis induced by different classes of chemotherapy-drugs in the rat. *Cancer Biol Ther*. 2006;5(9):1189-1195.
17. Fact Sheet – Tumor Markers. National Cancer Institute Web site. http://www.cancer.gov/cancertopics/factsheet/detection/tumor-markers. Accessed June 29, 2011.
18. Lutgens LC, Blijlevens NM, Deutz NE, Donnelly JP, Lambin P, de Pauw BE. Monitoring myeloablative therapy–induced small bowel toxicity by serum citrulline concentration: a comparison with sugar permeability tests. *Cancer*. 2005;103(1):191-199.
19. Logan RM, Gibson RJ, Sonis ST, Keefe DM. Nuclear factor-kappaB (NF-kappaB) and cyclooxygenase-2 (COX-2) expression in the oral mucosa following cancer chemotherapy. *Oral Oncol*. 2007; 43(4):395-401.
20. Gibson RJ, Cummins AG, Bowen JM, Logan RM, Healey T, Keefe DM. Apoptosis occurs early in the basal layer of the oral mucosa following cancer chemotherapy, *Asia-Pacific J Clin Oncol*. 2006;2(1):39-49.
21. Logan RM, Gibson RJ, Sonis ST, and Keefe DM. Oral mucositis – clinical presentation, histological features and proinflammatory cytokine expression. *Aust Dent J*. 2006;51:4.
22. Crosby WH, Kugler HW. Intraluminal biopsy of the small intestine: the intestinal biopsy capsule. *Am J Dig Dis*. 1957;2(5):236-41.
23. Keefe DM. *The effect of cytotoxic chemotherapy on the mucosa of the small intestine*. [MD thesis]. Adelaide, South Australia: University of Adelaide, Department of Medicine; 1998.

24. Keefe DM, Brealey J, Goland GJ, Cummins AG. Chemotherapy for cancer causes apoptosis that precedes hypoplasia in crypts of the small intestine in humans. *Gut*. 2000;47(5):632-637.
25. Lembcke B, Schneider H, Lankisch PG. Is the assay of disaccharidase activity in small bowel mucosal biopsy relevant for clinical gastroenterologists? *Klin Wochenschr*. 1989;67(11):568-575.
26. Lutgens LC, Deutz N, Granzier-Peeters M, et al. Plasma citrulline concentration: a surrogate end point for radiation-induced mucosal atrophy of the small bowel. *Int J Radiat Oncol Biol Phys*. 2004;60(1): 275-285.
27. Sutherland LR, Verhoef M, Wallace JL, Van Rosendaal G, Crutcher R, Meddings JB. A simple, non-invasive marker of gastric damage: sucrose permeability. *Lancet*. 1994;343(8904):998-1000.
28. Menzies IS, Laker MF, Pounder R, et al. Abnormal intestinal permeability to sugars in villous atrophy. *Lancet*. 1979;2(8152): 1107-1109.
29. Keefe DM, Cummins AG, Dale BM, Kotasek D, Robb TA, Sage RE. Effect of high-dose chemotherapy on intestinal permeability in humans. *Clin Sci. (London.)* 1997;92(4):385-389.
30. Melichar B, Kohout P, M. Brátová M, Solichová D, Králicková P, Zadák Z. Intestinal permeability in patients with chemotherapy-induced stomatitis. *J Cancer Res Clin Oncol*. 2001;127(5):314-318.
31. Daniele B, Secundulfo M, De Vivo R, et al. Effect of chemotherapy with 5-fluorouracil on intestinal permeability and absorption in patients with advanced colorectal cancer. *J Clin Gastroenterol*. 2001; 32(3):228-230.
32. Blijlevens NM, Lutgens LC, Schattenberg AV, Donnelly JP. Citrulline: a potentially simple quantitative marker of intestinal epithelial damage following myeloablative therapy. *Bone Marrow Transplant*. 2004;34(3):193-196.
33. Crenn P, Messing B, Cynober L. Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. *Clin Nutr*. 2008; 27(3):328-339.
34. Koetse HA, Stellaard F, Bijleveld CM, et al. Non-invasive detection of low intestinal lactase activity in children by use of a combined $^{13}CO_2/H_2$ breath test. *Scand J Gastroenterol*. 1999;34(1): 35-40.
35. Gilat T, Ben Hur H, Gelman-Malachi E, Terdiman R, Peled Y. Alterations of the colonic flora and their effect on the hydrogen breath test. *Gut* 1978;19(7):602-605.
36. Sategna-Guidetti C, Cruto E, Capobianco P. Breath hydrogen excretion after lactose and whole milk ingestion. A prospective comparison in lactase deficiency. *J Clin Gastroenterol*. 1989;11(3): 287-289.
37. Perman JA, Modler S, Olson AC. Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. *J Clin Invest*. 1981;67(3):643-650.
38. Vogelsang H, Ferenci P, Frotz S, Meryn S, Gangl A. Acidic colonic microclimate—possible reason for false negative hydrogen breath tests. *Gut*. 1988;29(1):21-26.
39. Welsh JD, Poley JR, Bhatia M, Stevenson DE. Intestinal disaccharidase activities in relation to age, race and mucosal damage. *Gastroenterology*. 1978;75(5):847-855.
40. Asp NG, Gudmand-Höyer E, Andersen B, Berg NO, Dahlqvist A. Distribution of disaccharidases, alkaline phosphatase, and some intracellular enzymes along the human small intestine. *Scand J Gastroenterol*. 1975;10:647-651.
41. Pelton NS, Tivey DR, Howarth GS, Davidson GP, Butler RN. A novel breath test for the non-invasive assessment of small intestinal mucosal injury following methotrexate administration in the rat. *Scand J Gastroenterol*. 2004;39(10):1015-1016.
42. Clarke JM, Pelton NC, Bajka BH, Howarth GS, Read LC, Butler RN. Use of the 13C sucrose breath test to assess chemotherapy-induced small intestinal mucositis in the rat. *Cancer Biol Ther*. 2006;5(1):34-38.
43. Tooley KL, Howarth GS, Lymn KA, Lawrence A, Butler RN. Oral ingestion of *Streptococcus thermophilus* does not affect mucositis severity or tumor progression in the tumor-bearing rat. *Cancer Biol Ther*. 2011;12(2):131-138.
44. Yazbeck R, Howarth GS, Borges L, et al. Non-invasive detection of a palifermin-mediated adaptive response following chemotherapy-induced damage to the distal small intestine of rats. *Cancer Biol Ther*. 2011;12(5):399-406.
45. Tooley KL, Saxon BR, Webster J, et al. A novel non-invasive biomarker for assessment of small intestinal mucositis in children with cancer undergoing chemotherapy. *Cancer Biol Ther*. 2006; 5(10):1275-1281.
46. Kaplowitz N. Biochemical and cellular mechanisms of toxic liver injury. *Semin Liver Dis*. 2002;22(2):137-144.
47. Vauthey JN, Pawlik TM, Ribero D, et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol*. 2006;24(13): 2065-2072.
48. Aloia TA, Fahy BN. Chemotherapy-associated hepatotoxicity: how concerned should we be? *Expert Rev Anticancer Ther*. 2010;10(4): 521-527.