

# Measuring tools for gastrointestinal toxicity

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## Purpose of review

The present review is timely owing to the previous paucity of biomarkers, particularly functional noninvasive tests, to evaluate the extent and severity of gastrointestinal toxicity in both animal models of chemotherapy and in cancer patients undergoing chemotherapy.

## Recent findings

The most recent findings using noninvasive functional biomarkers now allow longitudinal monitoring of the time course of damage and repair that occurs in the gastrointestinal tract following radiotherapy and chemotherapy. This monitoring has in turn enabled collection of objective evidence for efficacy of new antimucositis agents using animal models and, more importantly, for use in future randomized, double-blind, placebo-controlled clinical trials.

## Summary

In the past 12 months the  $^{13}\text{C}$  sucrose breath test has been applied to a series of animal models of chemotherapeutic damage, showing rapid monitoring of the efficacy of particular bioactive molecules is now possible at different stages of the damage and repair cycle. This biomarker has also been applied to childhood cancer studies of mucositis and now needs to be used in adult cancers for eventual adoption in routine clinical management of mucositis. An exciting possibility would be extension of the biomarker use to predict damage in other regions of the gastrointestinal tract, including oral mucosa.

## Keywords

intestinal function, intestinal permeability, noninvasive biomarkers, sucrose breath test

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## Introduction

Our understanding of the pathobiology of radiation and chemotherapy-induced injury to mucosal epithelium has undergone a conceptual change in the latter part of the past decade [1,2]. The recognition that not only the mucous epithelium lining the gastrointestinal tract is affected, but also the cells in the lamina propria, the musculature and the enteric nervous system can be impaired, created a new paradigm to begin to redefine iatrogenic damage during chemotherapy. The interactive loops set up during exposure, the genetic predisposition of the patient to particular anticancer drugs and the ensuing damage can all contribute to determine the impact and the severity of mucositis [3]. New measuring tools to detect and monitor this damage are needed.

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## Mucositis

Mucositis represents one of the most common side effects of chemotherapy. It can affect all or particular regions of the gastrointestinal tract with accompanying symptoms including stomatitis, dysphagia, dyspepsia,

diarrhoea, constipation and abdominal pain [4]. Mechanistically, different drugs and drug combinations, formulations and regimens induce damage and loss of barrier function in particular regions depending on the drug, its dose and the genetic predisposition of the patient. This loss of barrier function is generally associated with significant inflammation within the mucosa and systemically, which in turn manifests as ulceration in different regions of the alimentary tract.

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## Gastrointestinal toxicities

Gastrointestinal toxicities, particularly in the stomach, small intestine and colon, have not been easy to measure in patients with cancer undergoing chemotherapy or noninvasively in animal models [5,6]. This is partly due to the complex nature of tissue comprising the alimentary tract and to the relative inaccessibility of most regions of the gastrointestinal tract in patients undergoing chemotherapy, and also due to functional alterations of these components in response to a toxic insult. Moreover, the manifestations of acute versus chronic versus cumulative effects from frequent acute exposure to toxins,

such as in patients undergoing chemotherapy, potentially result in unique patterns and severity of damage. Breaching the barrier function of the intestine, either directly by toxic exposure or indirectly via a delayed inflammatory response, poses significant problems in designing tests to evaluate gut damage and dysfunction after toxic insult.

In the context of gastrointestinal side effects caused by chemotherapy, commonly called mucositis, the conceptual step forward provided by Sonis [7] – while initially based on information derived from the squamous epithelium lining the oral mucosa – is applicable to other mucous epithelia. This in part contributed to the development of recently updated clinical practice guidelines for the prevention and treatment of mucositis [8]. Therapy-induced mucosal damage is now thought to occur in five phases: initiation, up-regulation and message generation, amplification and signalling, ulceration, and healing.

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### **The primary target**

Notwithstanding the complexity and the phenotypic differences of different regions of the alimentary canal, the epithelium still remains the primary target for collateral damage during chemotherapy. Thus it is logical to devise reporter tests or biomarkers to establish the time course of damage and repair in the gastrointestinal tract. The present review focuses on some historical biomarkers but, more importantly, on more recent biomarkers that are now being used to provide noninvasive measures of the severity of mucosal damage, repair dynamics and possibly new ways to predict the mucositis risk.

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### **Role of the small intestine**

Arguably the small intestine is the organ of the gastrointestinal mucosa that is most susceptible to damage. The small intestine is pivotal for adequate nutrient uptake and for maintenance of energy metabolism in patients with cancer. The other organs comprising the alimentary canal primarily provide conduits for transfer of food and digesta (e.g. oesophagus), secretory activity (e.g. acid in stomach) and holding capacity for optimal delivery of digesta and uptake of water (stomach and colon, respectively). The small intestine, contrary to common belief, has thresholds for absorptive capacity [9] that can now be measured using recently designed noninvasive biomarkers, one of which will be described later in more detail. Clearly when the small intestine is damaged its primary functional activity is impaired. The functional activity of the small intestine is defined by the health of the villous/crypt unit, which is characterized by a balance between the mature compartment (villus) and the immature compartment (crypt). When damage occurs, therefore, it is reflected by a change in the activity of the brush border enzymes on the villus.

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### **Barrier function**

Intestinal permeability has historically been the primary measuring tool for assessing loss of barrier function due to chemotherapy. Most of the described tests target the small intestine, and in chemotherapy-induced damage the lactulose/rhamnose ratio and the lactulose/mannitol ratio have been the traditional noninvasive biomarkers. All systems and markers used suffer from deficiencies, and generally permeability is characterized by loss of tight junction patency between epithelial cells. The mechanistic nature of this ‘leakiness’ is still unclear but it has been reported to be more distinct in immature tissue (proliferative) than in mature (functionally absorptive) tissue [10,11]. The permeability measured relates largely to columnar cell organization rather than multi-layered mucosa such as that of the oral cavity, and can occur even when the epithelium is otherwise apparently undamaged. This may be why, in certain settings, intestinal permeability has not provided precise information of the state of health of the injured epithelium [12\*\*].

The tests that have been used measure permeability of different regions, and until recently this has not been easily discernable except for the small intestine. The D-xylose test was the first used, and various sugars and combinations of sugars and sugar alcohols have been used by various workers to try to describe the impairment of gastrointestinal tract barrier function. Whilst these tests have been useful they do not necessarily provide information on the state of functional health of different regions of the alimentary tract. <sup>51</sup>Cr-Labelled ethylenediamine tetraacetic acid is a pan marker of intestinal permeability but, owing to radioactivity, it has largely been confined to animal studies [13]. The chlorinated sucrose derivative sucralose [14], an artificial sweetener, has been used in other settings and is analogous to <sup>51</sup>Cr-labelled ethylenediamine tetraacetic acid.

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### **Sugar permeability tests**

The sugar permeability tests were first developed by Menzies *et al.* [15] to assess gut health in developing world populations where enteropathy is believed to be endemic. D-Xylose, 3-O-methylglucose, lactulose/rhamnose/mannitol/sucralose, lactulose/sucrose and various other combinations of differentially absorbed and metabolized sugars have been used. Primarily, the D-xylose permeability test and the lactulose/rhamnose test have been applied to chemotherapy-induced mucositis [16,17\*]. Some other combinations have been used, and have been moderately useful in settings where the loss of barrier function is severe [18]. The tests’ lack of ease of use and the realization that the most used tests only assess small intestinal leakiness and require urine or blood collections and high-performance liquid chromatography analysis, however, has

limited their adoption. Additionally, many factors and drugs can alter tight junction patency, potentially confounding interpretation [19,20].

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### Blood tests

Plasma citrulline measurements have been used most recently in myeloablative treatment regimens and appear to be indicative of mucosal mass, and it is suggested that this amino acid is unique to enterocyte metabolism. Plasma citrulline, however, does not appear to be useful as a biomarker for intestinal absorptive function in patients with short bowel syndrome [21<sup>••</sup>]. Combinations of plasma citrulline measurement and small intestinal permeability have been explored largely in haematological malignancies and in bone marrow transplant settings [22]. These markers deserve further study, particularly where the damage may be initially minor, to see whether this biomarker is sensitive enough to follow the potentially cumulative damage that may occur after multiple chemotherapy regimens.

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### Diarrhoea and constipation

Improved measurement of these symptoms partly resulting from chemotherapy-associated changes to the luminal environment helping to identify regional damage is becoming increasingly clear [23<sup>••</sup>]. There is still, however, an urgent need to design and validate noninvasive tests that can pinpoint the region(s) of damage to provide a mechanistic solution to explain the resultant dysfunction. The symptoms alone do not always allow this and they can be confounded by concomitant adjunctive therapy (e.g. codeine-included constipation). A further example is diarrhoea, which can be driven by secretory malfunction in the small intestine, osmotic overload or failure for adequate water absorption in the colon. Biomarkers of fermentation patterns [24] that can help to mechanistically separate the major source component for these symptoms need to be developed to provide better design of antimucositis agents.

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### Breath tests

Alkanes are expired in response to lipid peroxidation. An increased breath ethane level has been used as a marker of gastrointestinal inflammation [25,26]. Whilst this may be useful, taken alone the raised ethane levels may reflect inflammatory change in different regions of the gut but also in other affected organs (e.g. the lung). Combination of breath ethane with other biomarkers may be a very useful approach. Gastric motility and the transit time are changed when the gut is inflamed, and breath test biomarkers for these changes are readily available [27,28] that could be applied in chemotherapy-induced mucositis settings. Breath hydrogen tests for carbohydrate

malabsorption, particularly lactose malabsorption, are readily available. Lactase activity is lost or impaired in a high proportion of the global population. While the breath test for lactase deficiency can be useful and may detect damage to the small intestine, the high incidence of the underlying genetic deficiency precludes its use as a biomarker of small intestinal damage. In contrast, sucrase deficiency is a very rare condition ( $\ll 0.1\%$  of the population) and sucrase activity remains relatively stable throughout life, making it an ideal reporter of the functional health status of the small intestine [29].

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### <sup>13</sup>C sucrose breath test

The <sup>13</sup>C sucrose breath test (SBT) is a new concept for reporting on the status of health of the small intestinal villous. The test is based on the use of a selectively <sup>13</sup>C-labelled sucrose that enables a quantitative assessment of the absorptive capacity of the small intestine after ingestion of the stable isotope substrate, with an interval of collection of expired <sup>13</sup>CO<sub>2</sub> of 90 min. The cumulative percentage of the administered dose expired in a 90-min period is a marker of small intestinal damage and/or absorptive capacity. This level gives a quantitative indication of the status of small intestinal health, with a lower level indicating more impaired function [30<sup>••</sup>]. In contrast, the sucrose breath hydrogen test only measures the malabsorption of sucrose and is dependent on thresholds of sucrose absorption and on the type and extent of microflora to produce hydrogen as the breakdown product. These two factors are rate-limiting for this sucrose breath hydrogen test, which is reported as either malabsorption or adequate absorption. The SBT can be used in both animal models and in cancer patients to follow time courses of gut damage and repair with different drugs. This test now has been used in a number of animal models of damage [6,30<sup>••</sup>–32<sup>••</sup>,33<sup>•</sup>] and in assessment of potential agents for amelioration of mucositis as well as in a human study [12<sup>••</sup>]. The SBT now needs to be assessed in concert with apoptosis, diarrhoea and constipation, and so on [34], as a predictor of oral mucositis [35] and as a way to modify the stages of damage and repair. Cumulative or residual damage is very likely to occur in certain regimens and this can potentially be monitored by this test, allowing selective use of particular antimucositis agents (e.g. Palifermin).

A newer generation of noninvasive tests is also being developed that will allow pin-pointing of the regional damage and the severity of that damage. The SBT is used as the sentinel biomarker with selective permeability for different regions done at the same time [36]; for example, sucrose permeability with an abnormal SBT suggests both stomach and small intestine involvement, whereas sucrose permeability with a normal SBT indicates that the stomach is damaged whereas the small intestine is

not. This will provide selective modalities for assessing the side effects of newer targeted anticancer drugs and will also aid in the design of antimucositis agents and timing regimens for both classes of bioactives.

## Conclusion

The first challenge for any noninvasive biomarker of toxic insult to the gut is to provide a means to easily follow the stages of damage through to repair. The second imperative is to have a sufficiently sensitive test to pick up early damage and ultimately to be a predictive indicator of potential impending mucositis in different regions of the gut, from the mouth to the anus. The newer tests described in the present review provide this capability, particularly the SBT – which allows easy measurement of the functional health of the nutritionally important small intestine. The SBT has provided a means to mechanistically follow the stages of mucositis and its severity, which will in turn give us an evidence-based approach for designing better treatment regimens and for discovering new antimucositis agents. In the future, combinations of some of the described biomarkers for assessment of regional damage, provided they are practical, will further enhance our ability to prevent and treat mucositis.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 74–75).

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