Reviewer's report

Title: DNA index determination with Automated Cellular Imaging System (ACIS) in Barrett's esophagus: Comparison with CAS 200

Version: 2 Date: 10 April 2005

Reviewer: Thomas J Sebo

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General

This paper presents data on comparing data from two image analyzers (CAS 200 and Automated Cellular Imaging System [ACIS]) evaluating DNA content of 34 formalin-fixed, paraffin-embedded biopsies from 13 patients with specialized Barrett’s esophageal mucosa and varying degrees of atypia ranging from reactive to dysplasia (low and high grade) to adenocarcinoma. The authors concluded that, while there was strong correlation between the two devices, the ACIS instrument was more sensitive in detecting non-diploid nuclei than the CAS 200. Furthermore, the ACIS device was less time-consuming.

The paper is well-written and the approach in assessing these two instruments is, in general, sound. However, there are some major concerns this reviewer has which need to be addressed.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Abstract

1) The results section does not read well, in part, because of run-on sentences. First, there are no data presented to support the conclusion that ACIS is "less labor intensive." Second, I would recommend writing the latter part of this section as follows: There was a very good correlation between the DI values determined by ACIS and CAS 200. The ACIS was 25% more sensitive in detecting aneuploidy than CAS 200 with a mean DI 18% higher than CAS (p <0.001; paired t test).

2) I would recommend the authors add a sentence or two explaining how they defined "less labor intensive." If they defined this on the basis of the CAS 200 results being the "gold standard," then they should state as such.

Background

1) The authors need to define DI in the second paragraph, ie, "the DNA index (DI) of cancer cells is estimated." 

2) In the last paragraph of the Background, I would switch the order of the listed data findings, ie, (4) to (3) and (3) to (4). This makes the statements flow better.

Methods
1) In the Histology section, second paragraph, 3rd line, change â€œnumber of mitosisâ€ to read â€œnumber of mitoses.â€
2) The sentence on high grade dysplasia runs on and needs to be divided into more than one sentence. Either that or add an â€œandâ€ between â€œcryptsâ€ and â€œintraglandular.â€
3) It would be a good idea to not start sentences with abbreviations.
4) In the Aneuploidy analysis section, I am not sure what the authors mean by â€œaneuploid or tetraploid aneuploid.â€ Perhaps, a better term would be â€œaneuploid or non-diploid.â€
5) It appears from the discussion on CAS 200 and ACIS usage that the authors collected non-overlapping nuclear images for histogram generation. However, it is well-known that glandular lesions such as dysplasia in Barrettâ€™s or adenocarcinoma often have extensive nuclear overlap which can impact on the ability to obtain an accurate DNA histogram. The authors need to explain in better detail how they prevented this from causing the differences in the two instruments discussed in the Results section. For example, was ACIS able to capture nuclei better than CAS? If so, how?
6) Did the authors employ tissue correction to the DNA histograms prior to interpretation? If not, they need to explain why as it has been this reviewerâ€™s experience that uncorrected DNA histograms can miss 10% - 15% of non-diploid tumors.

Results

1) This may seem like a silly question, but the CAS 200 histogram photographs in Figure 2 remind me of the format used in the older CAS 100 analyzers. I wonder if some of the relative â€œinsensitivityâ€ in detecting non-diploid cells with the CAS system is, in part, related to the somewhat basic display of DNA content by the authorsâ€™ instrument. Perhaps, the authors could add a single sentence in the Methods or Results section commenting on this.
2) Table 1 is an important component of the authorsâ€™ study. However, the derivation of the data which suggests ACIS is superior in detecting higher DNA content is missing in the Methods section. For example, it is known that how an image analyzer technologist thresholds the digitalized nuclear images will potentially alter the DNA content results thereby changing the appearance of the DNA histogram. I think it would be extremely important for the authors to add some comment in the Methods section on how they made sure that thresholding issues between the two instruments did not impact on the final results. Furthermore, it would be useful for the authors to state how they made sure the exact same cells were analyzed for DNA content using the two instruments. These two aspects could easily impact on their findings.
3) In the Time required for one analysis section, the way the authors state their findings on time required to evaluate a specimen per instrument reads more anecdotal than scientific. It would be better if the authors would actually show a table or, at the very least, add a sentence or two on the exact means, medians, and ranges of time required for the technologists to collect data on each device.

Discussion

1) The authors begin their discussion by suggesting that DNA content may be a superior way of detecting early changes in pre-malignant/ malignant lesions than routine histologic examination. While this reviewer essentially agrees that most published data suggest DNA content analysis usually is more sensitive than routine cytology or histology in assessing for the presence of malignancy, this subject is by no means straightforward. Very early DNA alterations, which predate aneuploidy by image analysis may actually require a quantifiable nuclear morphometric evaluation to detect. In particular, single chromosome or locus-specific chromosomal abnormalities likely will not manifest as DNA indices of 1.5 or greater, but rather, normal or near-normal DNA indices between 1.0 and 1.2 (or less). Perhaps, the authors could add a sentence or two on this concept.
2) Along those same lines, the authors more or less are using the histologic interpretation of the biopsy specimen as the â€œgold standardâ€ for comparing CAS and ACIS. If that is the case, then how do we really know which instrument is more accurate? It strikes me that there is some
circular logic to this.

3) In some respects, the authors’ results can be viewed as alarming for the field of quantitative image analysis. If one presumes that all instruments, regardless of vendor, should be able to accurately and reproducibly quantify nuclear DNA content from individual, non-overlapped nuclei, how then do we explain the differences in the two instruments? If ACIS is more accurate, my first reaction to that would be that the exact same nuclei were not captured when using the two devices. If they were, then, as alluded to above, it strikes me as worrisome that two devices could generate different histograms from the exact same digitalized nuclear images.

4) Our laboratory recently embarked upon an evaluation of several different vendors’ instruments to see which new, state-of-the-art device would work best for our purposes. We processed all the slides identically using the same Feulgen stain and processing method. These slides were then compared with different analyzers including the ACIS. We found similar results comparing 5 different instruments. It seems to me that using different stained slides for DNA content assessment, at least in theory, introduces an error into the authors’ results which cannot easily be assessed. The authors should state why they chose different stains for the slides evaluated rather than use one Feulgen slide to compare the two analyzers. This easily could impact on the authors’ findings.

5) The authors’ explanations for higher DI values from ACIS are not completely valid. First of all, I agree that the stain used by the ACIS device may be more sensitive (thus, as mentioned above, the differences in the results may, in fact, be mainly due to the staining procedure and not the instruments). However, both CAS and ACIS use not only DNA content to capture nuclei but also morphometric features including nuclear area, shape, and density. Finally, no instrument necessarily requires a specific tissue thickness. The CAS, for example, has a tissue correction software which right-shifts DNA histograms based upon mathematical calculations regarding an estimate of how much depth or z-axis nuclear DNA content has been lost by cutting nuclei in tissue. I assume from the Methods section and Figure 2 that the authors did not employ such a tissue correction prior to interpreting the DNA histograms. This can result in under-calling upwards of 10%–15% of cases diploid when, in fact, they have small tetraploid or aneuploid peaks which escape detection. Conversely, the thicker the section of cut, the greater the nuclear overlap. This can lead to an inability to capture nuclei which are viewed by the technologist as abnormal. Finally, as we all know, setting the microtome at 5 or 7 microns does not guarantee that the tissue section of the glass slide will, indeed, be exactly 5 or 7 microns. Consequently, I don’t think this is an aspect of the study that can be controlled; as such, either not mentioning this or adding the statements I have included above would improve this part of the Discussion.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Tables and Figures

1) In general, whenever abbreviations are used in a table or figure, these should be explained in the table or figure legend. This will eliminate the need for the reader to hunt in the manuscript text for the definitions of the abbreviations.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the
major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests.