DNA Cytometry Testing for Cervical Cancer Screening – Response

We appreciate the interest of Drs. Garner, Guillaud, and MacAulay in our work and thank them for their critical comments on our recent publication (1). Early detection of cancerous lesions is a pivotal step for reducing cervical cancer-associated mortality. Finding a method which possesses high sensitivity and specificity in screening of mass populations has been difficult for cancer researchers. False negatives are a major problem for cervical cancer testing using conventional cytologic methods and frequent retesting is required (2). Previous studies have reported that DNA ploidy testing could increase the positive prediction of precancerous and cancerous lesions of uterine cervix compared with cytology (3–5). However, there has been little data on DNA ploidy cytometry testing as a single detection method in low-resource countries where it might be used for large population screening. This was the basis for performing the randomized study under discussion. Our goal was to test the hypothesis that DNA ploidy cytometry testing would be superior to conventional cytologic screening to detect early precancerous or cancerous cervical lesions.

First, we need to clarify the design of our study as to whether it was a randomized controlled trial or not. Garner et al. critically said that our study was not a randomized controlled trial because the data analyzed in our study were pooled together from both arms. In fact, we did perform both DNA cytometry and the cytologic evaluation on all patients’ specimens. This was done for ethical reasons because performing a single test on each arm would not have been approved by our institutional review boards. In Table 2 from our article, the results we reported included crossover screening. We apologize for not making this clear. However, all study processes were conducted in a randomized manner, i.e., patient’s eligibility screening, randomization, blindness concealment and uncovering, intention-to-treat, and per protocol analyses. Such analyses, at first, gave an overall understanding as to the two testing methods’ capability to detect possible cervical lesions. Furthermore, presenting the data in this manner did not influence the study design because this table only provided partial information about the whole study. We also performed analyses for the two separate groups (see Table 1). We felt we had made these points clear in the Materials and Methods and Results sections, but perhaps should have commented further in the Discussion.

As mentioned above, the data in our article was presented inconsistently between Table 2 and in the text, which resulted in our readers’ difficulty in understanding our design and data analyses. As Garner et al. pointed out, we stated that 54 cases of cancer were detected in the Results section, whereas Table 2 showed a total of 64 cases of cancer. As noted above, this is because Table 2 presented the results of crossover testing. The 54 cases stated in the Results section were the number of total cases detected by cytometry and cytology without crossover testing. Hence, crossover testing led to the detection of an additional 10 cancer cases. Although not reported, eight of these were when specimens from individuals in the cytology arm were screened by cytometry, and two were when specimens from individuals in the cytometry arm were screened by cytology. Thus, for purposes of data analysis, 32 cancers were found in the cytometry arm, and 22 cases were found in the cytology arm (see Table 1 of this response).

Regarding the sensitivity and specificity calculations in our study, we wish to clarify that our testing machines were corrected by the manufacturers with corresponding coefficients when doing mass screening. This was mainly due to the fact that our previous work had shown that they always had bias in detecting positive cases. The corrected coefficients are as follows: testing sensitivities of DNA cytometry and cytology are 1.011 and 0.436, respectively; and testing specificities are 1.0 and 0.837, respectively. Therefore, the sensitivities and specificities were as presented in our published article (for details regarding the calculating process, see the Supplementary File).

<table>
<thead>
<tr>
<th>Variable</th>
<th>DNA cytometry testing (n = 11,832)</th>
<th>Cytologic testing (n = 11,859)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of positive colposcopy</td>
<td>2,592 (78.9)</td>
<td>848 (74.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rate of positive HPV</td>
<td>306 (9.3)</td>
<td>112 (9.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rate of inflammation</td>
<td>231 (1.9)</td>
<td>86 (0.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rate of CIN grade 1</td>
<td>52 (0.4)</td>
<td>28 (0.2)</td>
<td>0.007</td>
</tr>
<tr>
<td>Rate of CIN grade 2 or 3</td>
<td>72 (0.6)</td>
<td>39 (0.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Rate of cancer</td>
<td>32 (0.3)</td>
<td>22 (0.2)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

NOTE: The number of patients in both groups including those that received only the assigned testing method, i.e., 46 women received DNA cytometry testing only and 48 women received cytologic testing only, were still included in this calculation.

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.
In our study, we provided detailed information of screening results stratified by age. Unfortunately, the subtotal calculations of positive results in the DNA cytometry group were incorrect due to an error in addition. Upon correcting this error and recalculating the values, we found that the rate of positive screening was 6,314 of 21,471 (29.4%), the rate of positive colposcopy was 5,167 of 6,314 (81.8%), and the rate of positive human papillomavirus was 712 of 6,314 (11.3%).

Another concern mentioned by Garner et al. was the 52 additional cases of cancer detected on re-screening and our failure to comment on this apparent high false negative rate for first screen. This was a mass screening study using methods modified from standard ones, hence, we chose not to discuss this issue. We would note that these 52 cases were detected using the primary detection approach in 2,191 women. There was crossover testing, but no additional cases were found. As can be gleaned from Supplementary Table S1, which includes these cases, cytometry detected 37 cancers and cytology detected 15 cases on re-screening.

Garner et al. also raised questions regarding how we achieved 100% sensitivity and 91.8% specificity when both methods were combined and used together. They stated that when combining the tests, either sensitivity or specificity would be decreased. We agree with this point. However, in our situation, all tested women with cancerous lesions of the cervix had undergone surgical procedures and the cervical specimens were sent for regular pathologic diagnosis. The pathology results confirmed the previous screening results in the DNA cytometry group, but in the cytology group, three women who were thought to have cancer were found to have precancerous lesions. Therefore, the combined sensitivities and specificities were calculated according to these final data.

Finally, regarding the definition of the DNA ploidy cytometry testing method, we incorrectly used the term "flow cytometry" in several sentences. We have contacted the Clinical Cancer Research editorial office and requested a correction of this issue, which the journal has agreed to do. Again, we thank Garner et al. for their critical reading and constructive comments on our article. This has provided us with an unanticipated opportunity to make a detailed clarification of issues that may have been considered by other readers. We hope we have been able to clarify the potential misunderstandings resulting from our incomplete presentation of the data and inadequate discussion.

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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
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