**Author's response to reviews**

**Title:** Keratocyte loss in corneal infection through apoptosis: a histologic study of 59 cases

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Reviewers Report

**General**

The authors of this study attempt to correlate keratocyte apoptosis with several infectious processes induced by fungal, bacterial, viral or Acanthamoeba keratitis in tissues from patients who underwent keratoplasty. They report keratocyte apoptosis independent of the infectious agent. There are several reports that have shown that damage of the epithelium releases inflammatory cytokines that induce rapid apoptosis in the stroma. In this study, apoptosis was found mainly in the posterior stroma, suggesting that there is some mechanism that prevents the keratocytes of this area from entering necrosis, possibly due to the inflammatory reaction. It would be interesting to pursue these findings using animal models. The authors are well aware that although their study reviewer a lot of samples from different cases, it is still very limited, in that the samples are taken from patients who underwent keratoplasty, therefore the tissue had already gone the route of irreversible damage.

**Major compulsory revisions:**

There are concerns with respect to the figures.

1. Figure 2 shows in high magnification H&E staining from a tissue with Acanthamoeba keratitis. Is the loss of keratocyte nuclei in the picture a result of apoptosis or necrosis? It would be helpful to show a low-magnification picture of the same section with TUNEL staining.

Figure 2 has been retained to demonstrate the presence of acanthamoeba cysts in the inflammatory-free zone in deeper stroma along associated with marked decrease in the keratocytes. However we have introduced another set of new figures - as 3 a & b which shows a case of Acanthameoba keratitis with inflammatory infiltrates in the superficial stroma and a quiet deeper stroma. The TUNEL stained section of the same shows diffuse staining of the inflammatory region with staining of few keratocytic nuclei in the deeper stroma.

The same has been explained in the text Page 8, line........

2. It is difficult to distinguish nuclear fragmentation in Figures 3 and 4. It would be more convincing to show photographs of different infections at lower magnification along with a control for comparison.

As explained above figure 3 has been replaced by figure 3a and 3b. In figure 4 we agree that the typical morphological appearance of fragmented nuclei is not highlighted in the TUNEL staining. It was seen either as diffuse staining or granular staining of the nuclei that are preserved. To substantiate this finding, we have added another figure (4b).

3. In addition, keratocytes close to the pathological foci appear to be normal. How do the authors explain this phenomenon?

We agree with the reviewers that there are some normal (unstained by TUNEL) nuclei in the areas surrounding the inflammatory zone. We had postulated and proved by TUNEL staining that apoptotic cell
death could explain the keratocyte loss observed in the stroma surrounding the inflammatory stroma in corneal infection. However the apoptotic cell death in the surrounding stroma but may or may not involve all the keratocytes.

4. The title is based on a hypothesis, not proof in this MS. What kind of protection occurred in the cases analyzed, if all the tissues had been replaced?

As the reviewers have rightly pointed out the protective role is purely a speculation, and therefore not appropriate for a title. We have changed the title to "Keratocyte loss in Corneal Infection through Apoptosis: a histologic study of 59 cases"

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

5. "Figure 7 must read "Figure 5"
Correction incorporated.

6. The tables are confusing e.g., Table 1 described 43 patients. The size of the ulcer is presented for 38 patients, the localization of the ulcer for 42 patient: does this mean that 42 of the 43 patients showed ulceration?

The total number of cases studied in this series was 59. However being a retrospective study, some of the clinical data could not be retrieved from medical records. The number in parenthesis represents actually the number of cases assessed for that finding.

7. In Table 2, keratocyte apoptosis, reduced - with respect to what?
It was meant to convey that the keratocyte number in the surrounding stroma was normal or reduced, compared to the expected number of keratocytic nuceli in a normal stroma. We agree that this was a subjective observation.

8. In Table 3, were the sizes of ulcers classified as small, medium or large? There are similar concerns for the other parameters measured. In "Keratocytes," there are three rows, what is the last.

We agree with the reviewers that the legends have not explained the table well. We attempted to correlate the clinical and histologic parameters (Eg. size of ulcer, stromal thinning etc) using Chi-square test and Fischer's exact test as shown in Table-3 Page 20. However as explained on Page 9 line 1-3 the correlation between apoptotic cell death and various types of infections could not be evaluated in view of high prevalence of positive staining in all types of infection. Other queries regarding ulcers and third row in "Keratocytes" has been corrected in the Table - 3 Page 20.