This study by Tsao and colleagues investigates the survival of neutrophils in cystic fibrosis lung transplant recipients with bacterial tracheobronchitis and suggests cleavage of annexin 1 as a marker of neutrophil necrosis. The study is of interest and the overall study design and data collection are straightforward and sound. However, I have some issues with the presentation and interpretation of the results.

Compulsory Revisions

1: My biggest issue overall is the interpretation of the results. According to the authors, annexin V positive cells are necrotic, however, most studies using this method conclude that annexin V positive cells are apoptotic. This is important as antimicrobial proteins are released in an unregulated manner during necrosis, but remain contained during apoptosis as described by the authors in the Background section. In addition, in the legend of Figure 4B the authors describe that the positive annexin V staining observed is a sign of apoptosis, however, in the results section the positive staining is interpreted as showing necrotic cells and is therefore confusing. In addition, the authors state necrosis as the fate of neutrophils in the infected airways in the abstract, but in the abstract, background and discussion they say oncotic, apoptotic and/or necrotic.

2: When the authors state that annexin 1 degradation, which according to their conclusions is associated with necrosis, may begin in the inflamed airways, it would be beneficial to discuss potential differences in function of healthy and CF neutrophils that could cause the observed variation. The authors should especially be aware of a publication by Moriceau et al. (2010) that illustrates impaired apoptosis of CF neutrophils and heterozygous carriers without inflammation.

3: Overall, the sample size used was quite small which makes the outcome of this study less robust and leaves room for subjective interpretation. Especially the Western blot results are only shown as images and were not quantified by densitometry which would give a more reliable way to compare samples. Additionally, for some experiments no number of repeats is given e.g. was flow cytometry only performed once or are results shown as representatives of n separate experiments (information of n should be included in the figure legends)?

4: I would like to see a comparison of neutrophil viability of healthy and CF
circulating neutrophils. The results from the flow cytometry analyses are designed to link protein degradation and necrosis, however, they were only performed for healthy circulating neutrophils. It is possible that protein degradation could be caused by something completely unrelated to necrosis e.g. something that is characteristic to CF (inflammation, etc...). Therefore, I recommend the addition of flow cytometry data for CF blood neutrophils to support the suggested conclusions.

Minor Essential Revisions
1: Change normal volunteers to healthy volunteers.
2: Remove commas before “and” as they are not needed.
3: The legends of Figure 1 and 4 should include some information of the imaging methodology used (e.g. light microscopy, original magnification, staining (with trypan blue))
4: It would be clearer for the reader if all abbreviations used in the figures are described in the figure legends, such as HS (healthy subject) and LTx (lung transplant recipient).
5: Abstract Methods: remove via “…was determined by morphologic appearance…”
6: Abstract Results: insert comma after “…particularly to a 33 kDa annexin 1 breakdown product (A1-BP)”
7: Abstract Conclusions: remove comma after tracheobronchitis
8: Background: remove comma after neutrophil-dominated (line 2)
9: Background: remove comma after “neutrophils undergo necrosis” (line 12)
10: Methods Isolation of BAL: explain abbreviation BAL and revise sentence in line 1
11: Bronchoalveolar lavage (BAL) fluids (BALF) were obtained from healthy (instead of normal) volunteers, patients with CF (remove comma) and clinically…”
12: Methods Isolation of BAL, line 12: rephrase and change “BALF cultures of BALF” to “bacterial (or microbial) cultures of BALF”
13: Methods Isolation of BAL, section 2, line 4: remove full stop after 10min
14: Methods Isolation of BAL, section 2, line 7: remove comma after cytospin slide preparation
15: Methods Isolation of BAL, section 2, line 8: remove triple space between full stop and “The rest of the cell…”
16: Methods Isolation of BAL, last line: remove comma after “…before use…”
17: Methods Cell culture: rephrase “About” to “On average 3x10^7 neutrophils were isolated from…”
18: Discussion, section 2, line 3: names of bacteria in italics and spell full name as abbreviation has not been explained elsewhere before
19: Figure legend 1: rephrase to read “Morphological analysis of neutrophils
isolated from peripheral blood from a healthy subject (HS PB) and a lung transplant recipient (LTx PB),...”

20: Figure legend 2: remove underlining of “BAL cells and peripheral cells”

21: Figure legend 2: remove “Right middle and bottom, same samples of Left meddle and bottom”, instead extend sentence in line 4: “Left middle and bottom (remove comma) lanes of HS1-HS3”

22: Figure legend 3: line 3 capital W for Western blot analysis

23: Figure 5B: remove typo in the label “control”

Discretionary Revisions

Figure 2 would benefit from rearranging some of the panels. It would be clearer for the reader if for example all neutrophil related Western blot panels appeared on the left with the actin control directly below the annexin 1 blot of peripheral cells and accordingly for macrophages on the right.

It would be interesting to know how many lung transplant recipients actually develop purulent bacterial tracheobronchitis (is it a common risk?) and if there was a difference between CF and other lung transplant recipients. So if this data is available it should be included for example in the discussion.

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests