Peer review file

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Reviewer A

Comment 1: SELECTION OF PATIENTS. According to data shown on Table 1, purulent appearance of pleural fluid was found in 60 patients (53.1% of the prospective arm of the multicenter study), which means that there were almost 47% of patients with no purulent pleural fluid and positive culture included in this series. Since I did not find any specific reference to this point (patient-selection) in the manuscript, I wonder if pleural fluid samples were routinely sent for culture in every patient, regardless the macroscopic aspect of the pleural fluid. If so, was a particular medium used for sample collection and transport to the Microbiology lab, including appropriate media for anaerobes?

Was a low pleural fluid pH considered as a suspicion of pleural infection in any case?

A paragraph containing this information should be included in the Methods section and it should also be commented in the Discussion.

Reply 1: We thank the reviewer for pointing this out. As a common practice in the departments participating in the present study, pleural fluid samples are routinely submitted for culture in every patient with pleural effusion of unknown etiology undergoing diagnostic thoracentesis regardless the clinical suspicion of pleural infection or the appearance of the fluid. All pleural fluid samples, regardless their appearance (purulent or not) or pH, were cultured using routine methodology, in blood culture bottles (for aerobic and anaerobic bacteria).

Changes in the text: We have appropriately updated the Methods (See page 7, lines 145-149 and page 8, lines 173-175) and Discussion (See page 12, lines 282-283) sections to incorporate and comment on the required information.

Comment 2: MANAGEMENT OF PATIENTS WITH CAPI. Were intrapleural fibrinolytics added in any case? If so, what type of fibrinolytics? Since this therapy has been reported to have a significant impact on patients' outcome, I believe that this information should also be included in the manuscript.

Reply 2: This is an extremely valid point. In this cohort, two patients received fibrinolytic therapy with tissue plasminogen activator (tPA) and deoxyribonuclease (DNase). Both patients recovered.

Changes in the text: We added some data about fibrinolytic therapy in the Results section (See page 10, lines 226-227)

Comment 3: OUTCOME.

- Was there any case with bronchopleural fistula? if so, how were those cases managed?

- What were the causes of death during hospitalization (29 patients)?

Reply 3: We thank the reviewer for giving us the opportunity to present these data too. In this study, there was no patient with bronchopleural fistula.

For all 29 patients, uncontrolled pleural sepsis was the cause of death.

Changes in the text: We have added details about the causes of mortality and if there was a case with bronchopleural fistula (See page 10, lines 224-225)

Reviewer B

Comment 1: Study appears to be a combination of retrospective and prospective. This has potential significant biases, including that only positive microbiological cases were recruited.

Reply 1: We thank the reviewer for giving us the opportunity to clarify this issue. The aim of the study was to identify the microbiology spectrum of pleural infection in Greece, a question that has not been studied before. In addition, we attempted to examine the drug-resistance patterns and identify factors associated with resistance. For all the above reasons, by purpose, we limited our observations in culture-positive patients.

As for combining retrospective and prospective groups, we need to make clear that the retrospective data were used only for descriptive purposes (to present the causative pathogens of pleural infection and the susceptibility to antimicrobial agents) to increase the size of the sample. All of the statistical analyses (univariate and multivariate), i.e the investigation for associations between clinical features and risk for death or antibiotic resistance were based on data from the prospective group only. We choose not to use the retrospective data for univariate and multivariate analysis, since the quality of the retrospectively obtain data were not optimal for this purpose.

Changes in the text: To avoid misunderstanding and make clear the features of our cohort, the phrase “culture-positive” was added at the title. To address the comment on the possible bias created by mixing the retrospective and prospective cohort for microbiological analysis, we rephrase the beginning of the paragraph on the limitation of the study, at Discussion session (See page 16, lines 368-375)

Comment 2: Within the abstract, the number of cases should be clearly stated, including a division between retrospective and prospective. This information appears to be lacking in the abstract.

Reply 2: We thank the reviewer for the comment.

Changes in the text: We have modified our abstract as advised (See page 3, lines 61-63, 64 and 66)

Comment 3: What is state that discovering the sensitivity of microbiological tests is a key outcome, but only include patients with a positive microbiological outcome? This does not quite make sense.

Reply 3: We thank the reviewer for making this comment and giving us the opportunity to clarify a central issue. We did not intent to examine the sensitivity of any method or test for identification of specific infective agents causing pleural infection. We focus on the microbiology and drug-resistance patterns of this infection in the Greek population and attempt to identify any risk factors of drug resistance and poor outcomes. To study the microbiology, we needed to include only patients with culture-positive effusions.

Changes in the text: We clarify this issue in the Discussion section (See page 13, lines 295-297)

Comment 4: The author should be clear on what their definition of hospital-acquired pleural infection was.

Reply 4: We thank the reviewer for his/her comment and practical advice on adding the definition of hospital-acquired pleural infection. We have addressed this (see the changes below).

Changes in the text: We have modified Methods section to present the definition of hospital-acquired and health-care-associated pleural infection (See page 7, lines 150-155).

Comment 5: - I do not think it is safe to combine prospective and retrospective cases, as the definitions and consistency will be different according to prospective and retrospective analysis. In fact, I would like to see a direct comparison of the main outcomes divided by prospective and retrospective data collection.

- Given there were no data available on important outcomes on the retrospective data collection, I think this should be entirely separated – in fact I think this does not really add to the data as it is only 45 patients. The criteria for selection etc… should be clearly stated for the retrospective patients.

Reply 5: We are grateful to the reviewer for commenting on this issue. The data of the retrospective group of the study were used only for descriptive purposes (to present the causative pathogens of pleural infection and the susceptibility to antimicrobial agents) to increase the size of the sample. All of the statistical analyses (univariate and multivariate), i.e the investigation for associations between clinical features and risk for death or antibiotic resistance were based on data from the prospective group only. We choose not to use the retrospective data for univariate and

multivariate analysis, since the quality of the retrospectively obtain data were not optimal for this purpose.

As far as the selection criteria of the retrospective group is concerned, these are the same as in the prospective.

Changes in the text: We have modified our text to discuss the issue of a possible bias because of the combination at Discussion section (See page 16, lines 368-375) and to clarify the selection criteria, at Methods section (See page 7, lines 156-158).

Comment 6: What was the definition of "antibiotic resistance"? Was this simply based on microbiological lab culture?

Reply 6: This is a quite important comment concerning antibiotic resistance. Antibiotic resistance is the ability of bacteria and other microorganisms to resist the effects of an antibiotic to which they were once susceptible. Multidrug resistance is defined as the resistance to at least one agent in three or more antimicrobial categories. In our study, the definition was simply based on microbiological pleural fluid culture.

Changes in the text: We have updated the text at Methods section, including the above definition (See page 8, lines 176-177).

Comment 7: The RAPID score has been derived, validated and prospectively assessed. It is gratifying to see that it is predictive in this dataset – but the additional parameters found to predict mortality (such as CRP) is driven by the data itself, as no validation on a different dataset has occurred. CRP and other parameters are therefore associated with mortality in this data – but this cannot be assumed to be the case in general, and this significant limitation must be clearly stated and discussed.

Reply 7: We are happy that the reviewer gave us the opportunity to comment more on this. In our study the RAPID score was revealed from the univariate and multivariate analysis as the most significant predictor of mortality. Univariate analysis provided evidence for CRP and diabetes as potentially significant predictors of mortality too. However, this observation needs further confirmation in large multicenter studies.

Changes in the text: We have changed the Discussion section (See page 16, lines 364-366) mentioning the need for confirmation of these findings in large studies.

Comment 8: Throughout, the authors should ensure to state that the results are "of positive isolates" – it would be entirely incorrect to suggest that this study has mapped the full microbiology of pleural infection, as micro negative cases were excluded by definition. This should also be reflected in the title and abstract.

Reply 8: We are grateful to the reviewer for helping us to improve the title of the manuscript and the presentation of our abstract. This study included only the patients

with culture- positive, community-acquired pleural infection and none with culture-negative one.

Changes in the text: We have modified the title and the abstract, highlighting that only cases with culture-positive pleural infection were included (See page 1, line 4 and pages 3-4, lines 57, 77).

Comment 9: There are very large studies assessing the entire microbiology of pleural infection in the world literature (Hassan et al, Cargil et al, ERJ) – these should be cited and discussed.

Reply 9: We thank the reviewer for his/her comment on adding and comment on discussion this large study concerning the microbiology of pleural infection.

Changes in the text: We added this large study on Discussion section (See page 13, lines 302-305 and page 14, line 309)

Comment 10: Table 5 – how was resistance to antibiotic combination determined? Was it treatment failure or in vitro work in the micro lab?

Reply 10: We thank the reviewer for pointing this out. The resistance to antibiotic combination was determined by the susceptibility test performed in each patient's pleural fluid culture. It was assumed that all patients received antimicrobial treatment for anaerobic bacteria in accordance with the current guidelines. Treatment failure was defined as in vitro failure according to the susceptibility tests of all isolates.

Changes in the text: We added details about how the resistance to antibiotic combination was determined on Methods section (See page 8, lines 176-181)

Reviewer C

Minor Comments

Comment 1: Could the authors reference the RAPID score ERJ publication DOI: 10.1183/13993003.00130-2020

Reply 1: Addressed

Changes in the text: We added this reference (See page 8, line 168 and page 15, line 354)

Comment 2: Figure 1, I would suggest reformatting the figure with black/white/grey colors and move sizing of the boxes to make visually better.

Reply 2: Addressed

Changes in the text: We modified this figure (See supplemental files)

Comment 3: Conclusion page 340 - common antibiotic regimens - although common in Greece the regimens are more advanced compared to other countries. E.g UK Could the authors comment on this statement?

Reply 3: This is a quite important comment concerning the common antibiotic regimens prescribed in Greece. It is true that these antibiotics and antibiotic combinations are more advanced than those prescribed in other countries i.e U.K. In our study, antibiotics and antibiotic combinations were included based on these local antibiotic policies. There is a growing use of broad-spectrum antimicrobial agents in Greece which has led to high levels of antimicrobial resistance. One reason for this practice is the lack of information of the microbiology of the pleural sepsis in the country, a problem that for the first time tries to address the study presented here.

Changes in the text: We included some data about the selection of antibiotics in our study in Conclusion section (See page 17, lines 394-395)