PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

Maintenance immunosuppressive therapy in liver transplantation: results from CESIT study, an Italian retrospective cohort study.

Authors

Bellini, Arianna; Finocchietti, Marco; Rosa, Alessandro Cesare; Masiero, Lucia; Trapani, Silvia; Cardillo, Massimo; Massari, Marco; Spila Alegiani, Stefania; Pierobon, Silvia; Ferroni, Eliana; Zanforlini, Martina; Leoni, Olivia; Ledda, Stefano; Garau, Donatella; Davoli, Marina; Addis, Antonio; Belleudi, Valeria; CESIT, Study Group

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VERSION 1 - REVIEW

Reviewer	**1**
Name	**Faitot, Francois**
Affiliation	**Les Hopitaux Universitaires de Strasbourg, HPB and Liver Transplantation Department**
Date	**16-Jun-2024**
COI	**None**

Comments to « Maintenance immunosuppressive therapy in liver transplantation : results from CESIT study, an Italian retrospective cohort study" by Bellini et all.

Bellini et al. investigate the evolution and impact of maintenance immunosuppression (IS) after liver transplantation (LT) from the analysis of public health data from 4 Northern Italian regions.

This is a well-written and original study that concludes that combination therapy associating tacrolimus and MMF or mTOR inhibitor has become more frequent and is associated with better survival than tacrolimus monotherapy.

The authors should be congratulated for the original method and the thorough analysis specifically that concerning changes of immunosuppression and its associated factors. The results are in perfect resonance with the real-life practice, underlining the value of retrospective large cohort based on crossed analysis of public health databases.

The 2 main questions that can be raised are the fact that IS strategy is clearly not the only factor affecting survival and the absence analysis of co-morbidities such as renal insufficiency. Three minor questions are regarding the use if mTOR inhibitors (mTORi), the heterogeneity of strategies among the 4 regions and the galenic of tacrolimus which is not discussed at all.

1/ The authors identify an evolution in IS strategy in time and better survival with combined IS strategy. One can believe that other improvements in LT patients care are associated with improvement in survival and that IS is not an independent risk factor for death. It could be interesting to have the evolution in survival according to time. Moreover, the authors should thoroughly discuss that point.

2/ In the discussion, the authors underline the main pitfall of the study which is the absence of analysis of significant parameters impacting the outcomes after liver transplantation notably extra-hepatic organ failure and most importantly renal failure. Together with cancer and cardiovascular diseases, it has become a major cause of mortality after LT and needs to be addressed. In line with the previous comment, this is the main drawback of the manuscript.

3/ The rate of mTORi seems quite low, if considering that 2/3 are transplanted for HCC and that renal insufficiency is quite frequent with the current increase of MELD scores at LT in recipients. There is an increase in the use of mTORi and quite a large difference according to regions. In our experience, mTORi use is much more frequent nowadays. Besides the inclusion criteria stops in 2019. Therefore, one could believe that the current practices are already very different from that described in the manuscript. Do the authors currently observe, in their practice, such a rise in mTORi or did it stop? What is the rate or mTORi use nowadays in the 4 regions?

4/ It seems that the IS strategy differ between the regions. It is well shared that each team has a specific strategy. This point should be raised in the discussion.

5/ Once-daily tacrolimus has shown significant benefits over twice-daily tacrolimus. This point is not discussed at all whereas it could probably be retrieved from the pharmaceutical dispensation records. Given the size of the population, this point could be of particular interest.

Although the manuscript is pleasant to read, it could be of help for the reader to have identified paragraphs in the Result section such as evolution, factors associated with combined IS therapy, survival.

In the same way, in Figure 1, it could be more interesting to present therapy options in order of decreasing frequency so that the reader immediately identifies the most common IS strategy.

Altogether, this is an original and well-constructed study that describes a large population of liver transplanted patients wisely using merged databases. It shows the decrease in use of

cyclosporine and the increased use of combined tacrolimus and MMF or mTORi with a beneficial impact on survival.

Reviewer	**2**
Name	**Piccinni, Carlo**
Affiliation	**Fondazione ReS (Ricerca e Salute) - Research and Health Foundation - CINECA partner**
Date	**17-Jun-2024**
COI	**None**

Dear Editor,

I read with great interest the article by Bellini and colleagues entitled "Maintenance immunosuppressive therapy in liver transplantation: results from CESIT study, an Italian retrospective cohort study" proposed as an original article for publication in BMJ Open.

The article is well written and clear in each part, with a balanced and coherent structure throughout. The STROBE checklist is addressed.

The article constitutes part of the CESIT study, which was supported by the Italian Medicines Agency among pharmacovigilance projects. Its objective was to generate real-world evidence (RWE) on the maintenance immunosuppression therapies post solid organ transplantation. The proposed study aimed to describe the pattern of usage of these therapies and to compare their efficacy and safety.

The topic of the study is of great importance, as there is a paucity of knowledge regarding the maintenance of immunosuppression after transplantation. The RWE could be a valuable solution to address this gap in knowledge. Furthermore, the study was based on Italian healthcare databases from four regions, utilising TheShinISS tool, which represents a potential solution to the numerous barriers to accessing these important data sources in Italy.

For these and other reasons, I believe that this article is suitable for publication in the BMJ Open. However, I have the following minor revision requests to improve the manuscript:

1. Abstract: Please explicitly define all acronyms at the first mention, as it is essential to understand the topic and to increase readability.

2. Strengths and limitations of this study. In the third bullet point, the authors stated that "medications prescribed in other regions were not considered." However, healthcare administrative databases allow for the association of a patient with all received prescriptions or dispensations, even if they occurred in regions different from the patient's resident one (so-called "mobilità passiva"). It is unclear why the authors did not consider these prescriptions.

3. Background (lines 98-99). The authors acknowledged the considerable heterogeneity in the therapeutic approach among transplant centres. While this is accurate, they should also emphasise that this variability can be mitigated by the establishment of specific care pathways, which are already well-developed in Italy (see Recenti Prog Med. 2024 Jun;115(6):267-270. doi: 10.1701/4274.42525.

4. Background (line 107). The acronym "MELD" should be explicitly defined and briefly described.

5. Material and Methods (line 135). The authors should provide further clarification regarding the semi-deterministic matching procedure employed to link healthcare administrative databases with the national transplant system, along with an explanation of the technical rationale behind this choice.

6. Material and Methods (line 152). The author used the prescription of "statins" as a proxy for alterations in lipid. It would be beneficial to understand why this approach was not extended to the wider class of lipid-lowering therapies.

7. Material and Methods (lines 161-162). It was unclear whether the switch/change in therapy was used as a censoring variable in the Cox model. Please provide more information on this.

8. Results (lines 172-180). Include p-values to better describe the differences observed in terms of therapeutic choices.

9. Results (lines 184-185). The authors elected to restrict the analysis of outcomes to patients treated with TAC-based therapy only. However, the decline in CsA over time does not negate the importance of analysing their outcomes. The authors should provide a more detailed rationale for this decision.

10. Discussion (line 255). Clarify that the integration interest is "clinical" data.

11. Discussion (lines 316-317). The authors should include a sentence outlining the necessity to overcome the current legal obstacles to data integration (in accordance with point 5). This is a highly pertinent topic.

12. Figure 1 (Therapy boxes). Please arrange the therapies in order of decreasing usage percentage.

VERSION 1 - AUTHOR RESPONSE

Reviewer: 1

Dr. Francois Faitot, Les Hopitaux Universitaires de Strasbourg

Comments to the Author:

Comments to « Maintenance immunosuppressive therapy in liver transplantation: results from CESIT study, an Italian retrospective cohort study" by Bellini et all.

Bellini et al. investigate the evolution and impact of maintenance immunosuppression (IS) after liver transplantation (LT) from the analysis of public health data from 4 Northern Italian regions.

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The authors should be congratulated for the original method and the thorough analysis specifically that concerning changes of immunosuppression and its associated factors. The results are in perfect resonance with the real-life practice, underlining the value of retrospective large cohort based on crossed analysis of public health databases.

The 2 main questions that can be raised are the fact that IS strategy is clearly not the only factor affecting survival and the absence analysis of co-morbidities such as renal insufficiency. Three minor questions are regarding the use if mTOR inhibitors (mTORi), the heterogeneity of strategies among the 4 regions and the galenic of tacrolimus which is not discussed at all.

- *1/ The authors identify an evolution in IS strategy in time and better survival with combined IS strategy. One can believe that other improvements in LT patients care are associated with improvement in survival and that IS is not an independent risk factor for death. It could be interesting to have the evolution in survival according to time. Moreover, the authors should thoroughly discuss that point.*

Thank you for the comment. We have added a section in the discussion where we talk about some other factors, besides immunosuppressive therapy, that may influence the improvement of survival in these patients. In this section, we refer to a report published in 2023 by the National Transplant Center. The report shows that the observed survival rate for the entire cohort of adult patients undergoing LT between 2000 and 2020 the one-year post-transplant is 87.2%, while at five years it is 75.8%. However, if we consider the more recent period from 2014 to 2020, the survival rate rises to 89.5% at one year and exceeds 90% in 2020, more than 10 percentage points higher than that observed in 2000.

- *2/ In the discussion, the authors underline the main pitfall of the study which is the absence of analysis of significant parameters impacting the outcomes after liver transplantation notably extra-hepatic organ failure and most importantly renal failure. Together with cancer and cardiovascular diseases, it has become a major cause of mortality after LT and needs to be addressed. In line with the previous comment, this is the main drawback of the manuscript.*

We are aware that this represents one of the main limitations of the work. The lack of data regarding renal function is certainly one of the major issues in the work we presented. We have emphasized this aspect in the section on study limitations.

- *3/ The rate of mTORi seems quite low, if considering that 2/3 are transplanted for HCC and that renal insufficiency is quite frequent with the current increase of MELD scores at LT in recipients. There is an increase in the use of mTORi and quite a large difference according to regions. In our experience, mTORi use is much more frequent nowadays. Besides the inclusion*

criteria stops in 2019. Therefore, one could believe that the current practices are already very different from that described in the manuscript. Do the authors currently observe, in their practice, such a rise in mTORi or did it stop? What is the rate or mTORi use nowadays in the 4 regions?

We believe it is very likely that the use of mTOR inhibitors is higher currently compared to the study period, especially considering the publication of recommendations by Cillo et al. in Italy, in 2020 (reference number 9 in the manuscript), which recommend the combination of TAC and mTOR inhibitors, particularly in patients with HCC. However, the CESIT study covered the period from 2009 to 2019, and we currently do not have data for the subsequent years. Given that transplant medicine is a constantly evolving field, we think it would be very interesting to repeat the analyses for the recent years as a development of this study.

- *4/ It seems that the IS strategy differ between the regions. It is well shared that each team has a specific strategy. This point should be raised in the discussion.*

We strongly agree; we have detected significant variability in the choice of therapeutic strategies among the various regions, and we believe that this could be related to the complexity of these patients, the many factors to consider in choosing a therapeutic combination, and the lack of consensus on the best therapeutic strategies for these patients. We have highlighted these aspects in the discussion section. Additionally, prior to this work, we had published the following article as part of the CESIT project:

Marino ML, Rosa AC, Finocchietti M, et al. Temporal and spatial variability of immunosuppressive therapies in transplant patients: An observational study in Italy. Frontiers in Transplantation. 2023;1.

- *5/ Once-daily tacrolimus has shown significant benefits over twice-daily tacrolimus. This point is not discussed at all whereas it could probably be retrieved from the pharmaceutical dispensation records. Given the size of the population, this point could be of particular interest.*

Thank you for the comment. We investigated part of this aspect in the first article published -within the context of the CESIT project (*Belleudi V, Rosa AC, Finocchietti M, et al. An Italian multicentre distributed data research network to study the use, effectiveness, and safety of immunosuppressive drugs in transplant patients: Framework and perspectives of the CESIT project. Front Pharmacol. 2022;13.*), in which the proportion of liver transplant recipients using immediate-release TAC was reported (31%). Additionally, in another recently published work (*Finocchietti M, Marino ML, Rosa AC, et al. Immunosuppression with Generics in Liver and Kidney Transplantation: A Real-World Evidence Study. Drug Des Devel Ther. 2024;18:53-69. Published 2024 Jan 12. doi:10.2147/DDDT.S431121*), we highlighted that in the liver cohort, there was a significant proportion (55.1%) of patients who switched from the generic to the branded formulation. We explained this by noting the high use of once-daily TAC (not available in generic form).

At the moment, we have not conducted an efficacy and safety analysis comparing the two formulations of TAC, but we consider it a very interesting point and a potential future development of the work.

- *Although the manuscript is pleasant to read, it could be of help for the reader to have identified paragraphs in the Result section such as evolution, factors associated with combined IS therapy, survival.*

Thank you for the suggestion, we have divided the results section into four paragraphs:

1. Cohort Selection and Use of immunosuppressive therapies over time;
2. Characteristics of the cohort;
3. Effectiveness and safety analysis; 4
4. Switches of immunosuppressive therapies during follow up

- *In the same way, in Figure 1, it could be more interesting to present therapy options in order of decreasing frequency so that the reader immediately identifies the most common IS strategy.*

Thank you, we have modified Figure 1 as suggested.

Altogether, this is an original and well-constructed study that describes a large population of liver transplanted patients wisely using merged databases. It shows the decrease in use of cyclosporine and the increased use of combined tacrolimus and MMF or mTORi with a beneficial impact on survival.

Reviewer: 2

Dr. Carlo Piccinni, Fondazione ReS (Ricerca e Salute) - Research and Health Foundation - CINECA partner Co wrote with Letizia Dondi, Fondazione ReS (Ricerca e Salute)

Comments to the Author:

Dear Editor,

I read with great interest the article by Bellini and colleagues entitled "Maintenance immunosuppressive therapy in liver transplantation: results from CESIT study, an Italian retrospective cohort study" proposed as an original article for publication in BMJ Open.

The article is well written and clear in each part, with a balanced and coherent structure throughout. The STROBE checklist is addressed.

The article constitutes part of the CESIT study, which was supported by the Italian Medicines Agency among pharmacovigilance projects. Its objective was to generate real-world evidence (RWE) on the maintenance immunosuppression therapies post solid organ transplantation. The proposed study aimed to describe the pattern of usage of these therapies and to compare their efficacy and safety.

The topic of the study is of great importance, as there is a paucity of knowledge regarding the maintenance of immunosuppression after transplantation. The RWE could be a valuable solution to address this gap in knowledge. Furthermore, the study was based on Italian healthcare databases from four regions, utilising TheShinISS tool, which represents a potential solution to the numerous barriers to accessing these important data sources in Italy.

For these and other reasons, I believe that this article is suitable for publication in the BMJ Open. However, I have the following minor revision requests to improve the manuscript:

- *Abstract: Please explicitly define all acronyms at the first mention, as it is essential to understand the topic and to increase readability.*

Thank you for the suggestion, we have included the definitions of the acronyms in the abstract as well.

- *2. Strengths and limitations of this study. In the third bullet point, the authors stated that "medications prescribed in other regions were not considered." However, healthcare administrative databases allow for the association of a patient with all received prescriptions or dispensations, even if they occurred in regions different from the patient's resident one (so-called "mobilità passiva"). It is unclear why the authors did not consider these prescriptions.*

Thank you very much, we agree with you that it would have been interesting to analyze, but, as part of the CESIT Project, data on "mobilità passiva" were not requested. We had access exclusively to the drug prescriptions made within the region of residence. However, to mitigate this limitation, we excluded from the study all transplant recipients not residing in the regions under study.

- *3. Background (lines 98-99). The authors acknowledged the considerable heterogeneity in the therapeutic approach among transplant centres. While this is accurate, they should also emphasise that this variability can be mitigated by the establishment of specific care pathways, which are already well-developed in Italy (see Recenti Prog Med. 2024 Jun;115(6):267-270. doi: 10.1701/4274.42525.*

During the years of the study, the data did not reveal the implementation of specific care pathways for patients undergoing liver transplants. However, we agree that formulating specific diagnostic and therapeutic pathways is certainly one way to reduce variability in patient care. We have included a reference to this aspect in the discussion section, where we comment on the high variability of therapies.

- *4. Background (line 107). The acronym "MELD" should be explicitly defined and briefly described.*

Thank you, we have added the requested information.

- *5. Material and Methods (line 135). The authors should provide further clarification regarding the semi-deterministic matching procedure employed to link healthcare administrative databases with the national transplant system, along with an explanation of the technical rationale behind this choice.*

The linkage procedure between the national transplant system and the healthcare administrative databases is explained in detail in a previous work published by the CESIT group (*Belleudi V, Rosa AC, Finocchietti M, et al. An Italian multicentre distributed data research network to study the use, effectiveness, and safety of immunosuppressive drugs in transplant patients: Framework and perspectives of the CESIT project. Front Pharmacol. 2022;13*.). The following is reported in that work:

"To link this information system with the transplant cohort, an ad hoc stepwise deterministic record linkage procedure has been defined using pseudonymous information (e.g., sex, organ type, year and month of birth, year and month of transplant, and transplant's hospital), which is compliant with legislation on data protection and privacy and the principle of data minimization in analytical dataset creation. The anonymous record linkage approach allows an exact match on a pre-processed subset of personal identifiers. These identifiers are concatenated and encoded into a 'key,' which can identify an individual. Subjects with duplicated keys are removed to perform the linkage procedure. Sensitive

information fields used in the procedure but not needed for the study are not reported in the analytical dataset."

We have referenced this article in the materials and methods section and included additional details.

- *6. Material and Methods (line 152). The author used the prescription of "statins" as a proxy for alterations in lipid. It would be beneficial to understand why this approach was not extended to the wider class of lipid-lowering therapies.*

Thank you for pointing this out. It is a nomenclature error. Although we referred only to statins, we considered in all analyses all prescriptions with ATC code C10, which includes all lipid modifying agents. The error has been corrected in the text and figures.

- *7. Material and Methods (lines 161-162). It was unclear whether the switch/change in therapy was used as a censoring variable in the Cox model. Please provide more information on this.*

The efficacy and safety analysis is an Intention-To-Treat analysis, whit the follow-up period extending from day 31 after the discharge date up to a maximum of 5 years. The data are censored for death, end of study date, end of follow-up (5 years), or loss to follow-up. For the production of Figures 4a and 4b, we instead considered and quantified the different switches made by patients in the cohort during the follow-up*.*

- *Results (lines 172-180). Include p-values to better describe the differences observed in terms of therapeutic choices.*

We added p-values as suggested.

- *9. Results (lines 184-185). The authors elected to restrict the analysis of outcomes to patients treated with TAC-based therapy only. However, the decline in CsA over time does not negate the importance of analysing their outcomes. The authors should provide a more detailed rationale for this decision.*

We have expanded the sentence in the results section to explain the reasons for this choice. We decided not to include CsA-based therapies in the efficacy and safety analysis because the number of CsA users was too small to obtain stable estimates. Additionally, given that many recent publications have demonstrated the superior efficacy and risk profile of TAC compared to CsA (lines 237-241) and recommend limiting the use of CsA to specific cases, we believed it would be more interesting to focus on evaluating any differences between TAC-based regimens.

- *10. Discussion (line 255). Clarify that the integration interest is "clinical" data.*

Thank you for the suggestion, we have modified the sentence as follows: "From the efficacy and safety analysis, it emerged that TAC-monotherapy was associated with an increases risk of mortality in the cirrhosis sub-cohort. This finding did not correspond with an increased risk of rejection, infections or MACE. A possible explanation could be related to the nephrotoxic effects of CNI inhibitors, leading to renal dysfunction in these patients. As previously mentioned, this clinical information could not be traced from the administrative data in our study."

- *11. Discussion (lines 316-317). The authors should include a sentence outlining the necessity to overcome the current legal obstacles to data integration (in accordance with point 5). This is a highly pertinent topic.*

We agree and have added considerations on this topic in the discussion section (lines 352-355). We particularly emphasize how the CESIT project facilitated the integration of data on immunosuppressive treatments from various regions and sources through TheShinISS. This represents an important strength of the work, especially in light of the new privacy regulations and the associated challenges in aggregating information from different data sources.

- *12. Figure 1 (Therapy boxes). Please arrange the therapies in order of decreasing usage percentage.*

Thank you, we have modified Figure 1 as suggested.

VERSION 2 - REVIEW

Reviewer	**1**
Name	**Faitot, Francois**
Affiliation	**Les Hopitaux Universitaires de Strasbourg, HPB and Liver Transplantation Department**
Date	**13-Aug-2024**
COI	**None**

Bellini et al. submit a revision for their manuscript entitled "Maintenance immunosuppressive therapy in liver transplantation: results from the CEST study, an Italian retrospective cohort study".

As already stated in the first comments, the authors should be congratulated for the design of the study, the original idea and the analysis of the data they initially took in consideration.

However, the revisions are only ones regarding the form and not the content. Specifically, the relation between Tac-monotherapy and survival, although observed in our current practice, is not sufficiently supported by the reported data. Refering to a published report about survival evolution is not sufficient to link, in the studied population, changes in immunosuppressive strategy to survival, mainly because many other factors may have influenced the long-term results.

Hence, based on the herein reported data, it is only possible to describe the evolution in IS regimens but not to conclude in its impact on survival.

Given the evolution in IS regimen practices that the authors aknowledge, one may question the added value of the manuscript even though its method and the size of cohort should be put forward.

Reviewer   2

Name	**Piccinni, Carlo**
Affiliation	**Fondazione ReS (Ricerca e Salute) - Research and Health Foundation - CINECA partner**
Date	**23-Aug-2024**
COI	**None**

In the revised version of the manuscript, the authors have addressed all of my requests and those of the other reviewer. In my opinion, the work can be accepted for publication. I would like to extend my congratulations to the authors on this manuscript.

VERSION 2 - AUTHOR RESPONSE

Reviewer: 1
Dr. Francois Faitot, Les Hopitaux Universitaires de Strasbourg
Comments to the Author:
Bellini et al. submit a revision for their manuscript entitled "Maintenance immunosuppressive therapy in liver transplantation: results from the CEST study, an Italian retrospective cohort study".
As already stated in the first comments, the authors should be congratulated for the design of the study, the original idea and the analysis of the data they initially took in consideration. However, the revisions are only ones regarding the form and not the content. Specifically, the relation between Tac-monotherapy and survival, although observed in our current practice, is not sufficiently supported by the reported data. Refering to a published report about survival evolution is not sufficient to link, in the studied population, changes in immunosuppressive strategy to survival, mainly because many other factors may have influenced the long-term results.
Hence, based on the herein reported data, it is only possible to describe the evolution in IS regimens but not to conclude in its impact on survival.
Given the evolution in IS regimen practices that the authors aknowledge, one may question the added value of the manuscript even though its method and the size of cohort should be put forward.

Dear Reviewer,

Thank you for appreciating our rigorous methodology. We understand your concerns about the interpretation of our study results and acknowledge that, given the type of data available and the information at our disposal, it is not possible to draw definitive conclusions about the impact of tacrolimus monotherapy on the survival of liver transplant patients. We implemented the most appropriate analysis with the available data; however, as you and the discussion have pointed out, residual confounding may still exist, and we are limited by unmeasured data.

To strengthen these aspects, we have added further paragraphs to the discussion in the new version of manuscript, where we clarified the role of "residual confounding" in limiting the study's conclusions. We also emphasized the complex interplay of factors affecting survival,

including but not limited to immunosuppressive therapy. Moreover, we have modified the conclusions as follows: "Moreover, a potential association between TAC-monotherapy and increased mortality in the cirrhosis cohort was identified, although more detailed data would be necessary to evaluate the absolute impact of immunosuppressive therapy on survival and other outcomes."

Nevertheless, we believe that, considering the large number of patients involved and the methodological rigor of the study, this work can provide valuable insights into the use of immunosuppressive therapies in real-world clinical practice and highlight possible associations between pharmacological treatments and the outcomes considered

We trust these revisions enhance the clarity of our work and hope the new version of the manuscript meets your requirements.

Reviewer: 2
Dr. Carlo Piccinni, Fondazione ReS (Ricerca e Salute) - Research and Health Foundation - CINECA partner
Comments to the Author:
In the revised version of the manuscript, the authors have addressed all of my requests and those of the other reviewer. In my opinion, the work can be accepted for publication. I would like to extend my congratulations to the authors on this manuscript.

We thank the reviewer for the feedback.

Reviewer: 1
Competing interests of Reviewer: None

Reviewer: 2
Competing interests of Reviewer: None