Peer Review File

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REVIEWER A

Methods

Comment 1: *In the two groups, did the same surgical resident perform the surgical procedure? If not, is there a big inconsistency regarding background information between surgeons? What is their level of experience?*

Reply 1: Thank you for the question. All laparoscopic cholecystectomies were performed by residents in their third and fourth year of training. Thus, there was a comparable level of experience.

Changes in the text: "All laparoscopic cholecystectomies were performed in French position by surgical residents in their third or fourth year of residency under supervision of one out of five consultants, with an experience of at least 200 cholecystectomies. These were the same residents and consultants in both groups."

Comment 2: *Line 134, please add more details about the "Laboratory values", such as aspartate aminotransferase, c-reactive protein, gamma-glutamyl transferase etc.*

Reply 2: Thanks for the comment. We have listed the laboratory values in the material and methods section, subheading "Laboratory values"

Changes in the text: "Alanine and aspartate aminotransferase (ALT, AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase were identified as possible markers for liver damage. White blood count and c-reactive protein were used as markers for inflammation. Laboratory values were obtained in all patients on the first preoperative day and on the first and third postoperative days (POD)."

Comment 3: *Is there any missing data in the conventional group, since it was retrospectively retracted?*

Reply 3: There were no missing data in the retrospective control group with 4-port cholecystectomy.

Changes in the text: Methods: "In retrospective group 2 there were no missing data in comparison to prospective group 1 (parameters see below)."

Comment 4: *Move the "Primary and secondary outcomes" section to the top of "Statistical analysis" section. Further, suggest the authors merge the "Primary and secondary outcomes" into the "Data acquisition and collection section". This would also avoid some lengthy statements.*

Reply 4: Thank you for this recommendation. We have changed the order of the headings and text in the Methods section. This has made this part much clearer and easier to understand.

Changes in the text: The methods section has been reorganized, including new headings.

Comment 5: *Very high proportion of participants left the study (60%). What happened to these patients- were they excluded, or crossed over to the control group?*

Reply 5: Thank you for this very important question. We did not explain this sufficiently in the first draft of the manuscript. LiVac patients who were intraoperatively switched to conventional 4-port cholecystectomy were still evaluated as LiVac patients. This was to avoid any "attrition bias." In all LiVac-

patients with a change of the surgical procedure, vacuum was applied to the liver at least temporarily.
Changes in the text: Methods, study design: "If the application of the LiVac retractor was unsuccessful in patients of group 1, the procedure was switched to the standard 4-port technique. To avoid attrition bias, these patients were not excluded, but still evaluated as LiVac patients."

Comment 6: *Are there any adverse events in the conventional 4-port laparoscopic cholecystectomy group?*
Reply 6: No, there were no adverse events in the conventional 4-port laparoscopic cholecystectomy group.
Changes in the text: no changes in the text were made regarding this question.

Results
Comment 7: *The authors only present the number of participants in the FINAL stage. We suggest the authors use a flow diagram to report the number of participants of the two groups at each stage, from the selection of potential eligible ones to the final included ones, and with reasons for exclusion.*
Reply 7: Thank you for this great recommendation. We have created a figure (flowchart) showing the patient selection and final analysis.
Changes in the text: see figure 1 (flowchart)

Comment 8: *The subheadings of the Results section should be replaced with "Primary outcomes" and "Secondary outcomes", which will inform the readers much better.*
Reply 8: Thank you for this good advice. The subheadings in the results section have been changed.
Changes in the text: We changed the subheadings in the results section ("primary outcomes" and "secondary outcomes").

Comment 9: *Please present all key results with precise data and their precisions, instead of vague wording like "no significant differences". For example, the data shown between lines 195 and 199.*
Reply 9: You are absolutely right, the presentation of the results in the text was not adequate.
Changes in the text: We added the key results with precise data in the results section according your advice.

Comment 10: *Another drawback of the study appears to be the application of parametric tests. Normal data distribution in such a small group seems unlikely. The authors could firstly perform the normality test. For example, in Table 2, the values of POD 1 and POD 3 may be skewed distribution. The publication may require statistical re-analysis using non-parametric tests.*
Reply 10: Thank you very much for this important note. We have conducted a statistical re-analysis. It was found that not all conditions for the (non-parametric) Mann-Whitney U test were met for five variables. We have described this in the Results and Discussion sections. In addition, we have marked these variables in the tables, and added a corresponding explanation. The detailed statistical review is attached below (pages 7-14 of the response letter)

Changes in the text: Results, secondary outcomes: "The following parameters showed a deviation in the distribution of values: baseline ALT, baseline CRP, NRS on POD 1, ALP on POD 1 and AST on POD 3. Thus, for these parameters one prerequisite for the Mann-Whitney U test was not met."
Discussion: "For the parameters baseline ALT, baseline CRP, NRS on POD 1, ALP on POD 1 and AST on POD 3, not all the necessary prerequisites for performing the Mann-Whitney U test were fulfilled, so that these test results should be interpreted with particular caution."

Comment 11: *Since 15 cases in the LiVac group switched to 4-port LC, should the numbers in LiVac 3-port LC group in Table 2 be changed to 10?*
Reply 11: Again, we would like to apologize for this ambiguity. We have not described the evaluation sufficiently. The number of LiVac patients remained unchanged because even after switching to laparoscopic 4-port cholecystectomy, they didn´t change to the other group, nor were they excluded. The reason for this was to avoid an attrition bias.
Changes in the text: As described above, we tried to explain the evaluation more precisely: methods, study design: "If the application of the LiVac retractor was unsuccessful in patients of group 1, the procedure was switched to the standard 4-port technique. To avoid attrition bias, these patients were not excluded, but still evaluated as LiVac patients."

Comment 12: *Some inappropriate places appeared in the tables. In Table 1, "Age ± SD [years]" should be "Age [years] (Mean± SD)". Please revise the presentations of all continuous variables in the first column of the tables. For all the laboratory values, "27.6 [20.1]" should be "27.6±20.1". Please check through the tables to address similar concerns.*
Reply 12: Sorry for these formal and unprofessional errors.
Changes in the text: All data presentations in tables were revised according to your advice (tables 1 and 2).

Comment 13: *The decimal places of all values should be consistent in the tables and the main text.*
Reply 13: You are absolutely right – values have to be consistent.
Changes in the text: All values were checked for consistency and revised when necessary.

Comment 14: *Efforts are still needed in improving the quality of Figures.*
Reply 14: Thank you – we think that you are talking about the intraoperative figures which are indeed not of optimal quality.
Changes in the text: The intraoperative figures were replaced by figures of better quality (figures 4 and 5).

Comment 15: *Please kindly revise the P value in the tables and the main text:*
-If the P<0.001, report "P<0.001".
-If the P value is between 0.001 and 0.01 and less than 0.01, report the specific P value to 3 decimal places, e.g., "P=0.001" "P=0.009".
-If the P≥ 0.01, report the specific P-value to 2 decimal places, e.g. "P=0.01" "P=0.06" "P=0.10" "P=0.90".

-If the P-value is greater than 0.99, report "P > 0.99".
-Do not round P-values, do not report "not significant" simply because the data are greater than an arbitrary value, and do not report only vague bounds such as P<0.05, as described above, but report the exact P-value.
-P value in the tables should be consistent with that in the Results section.
Reply 15: Thank you for the comment on the correct reporting of p-values.
Changes in the text: We revised all p-values according to your recommendation.

REVIEWER B
Comment 1: *Could you please clarify what elective cholecystectomy means at your institution? I assume it is for patients with previous episodes of biliary colic and no previous cholecystitis treated with antibiotics, or percutaneous tube drainage? If so, was there a difference between the two groups maybe affecting the selection in group 1?*
Reply 1: Thank you for pointing this out. Only patients with episodes of biliary colic and without preceding cholecystitis were included in the study. We have clarified this in the method section.
Changes in the text: Methods; Inclusion and exclusion criteria: "Only patients with elective cholecystectomy for symptomatic cholelithiasis (e.g. previous episodes of biliary colic) were included. A minimum age of 18 years and voluntary informed consent were required for inclusion in the study.
Exclusion criteria were the presence of acute or previous cholecystitis, a body mass index (BMI) > 35 kg/m^2, and an ASA (American Society of Anesthesiologists) score ≥ 4."

Comment 2: *From your discussion, it appears using the 3 port LiVac requires the operator to be skilled laparoscopically. Therefore, can you comment on the consultant surgeons experience with cholecystectomy between the groups? Were the surgeons willing to try the 3 port LiVac more laparoscopically inclined than those who chose not to try the LiVac system during the study period?*
Reply 2: This is a very important point. All consultants were experienced laparoscopic surgeons with at least > 200 laparoscopic cholecystectomies. There was no difference between the groups in this regard. There were no reservations among the consultants about the trial of the LiVac retractor.
Changes in the text: Methods, Medical treatment procedures: "All laparoscopic cholecystectomies were performed in French position by surgical residents in their third or fourth year of residency under supervision of one out of five consultants, with an experience of at least 200 cholecystectomies. These were the same residents and consultants in both groups. "

Comment 3: *Is it standard at your institution to keep patients in hospital for 3 days postoperatively for elective cholecystectomy? Many institutions would send elective cholecystectomy home on the same day unless there are some patient factors or surgeon concerns to keep the patient in hospital. This brings the question, for your retrospectively selected group 2, did they have more difficult operations or something else explaining why they remained in hospital for 3 days post operatively? Perhaps the complications would be even less for group 2 if your protocol did not limit to those who remained in hospital for 3 days.*

Reply 3: Thank you for this question. Indeed, it was standard in our hospital at that time period that patients with laparoscopic cholecystectomy were hospitalized for at least three days. Of course, you are right that there is no valid medical justification for this standard, and we now make the length of inpatient stay dependent on the course of the operation (e.g. intraoperative complications).
Changes in the text: Methods, Study design: "Length of hospital stay was as a standard at least three days for all patients undergoing laparoscopic cholecystectomy during the study period. Thus, a three-day follow-up (complications, pain levels, laboratory values; see below) was possible for all patients."

Comment 4: *Can you please briefly comment on the type of injury requiring intervention for the grade 3 Clavien-Dindo complications for both group 1 and group 2?*
Reply 4: Thank you - we have added the specific grade 3 complications of all patients.
Changes in the text: Results, primary outcome: "In the LiVac group, tangential injury to the CBD was detected in one patient (4 %) postoperatively. This patient underwent ERCP with stent placement and re-laparoscopy with irrigation and drainage placement, and recovered completely thereafter.
In group 2, two patients (4 %) required re-laparoscopy (irrigation and drainage placement in each case, once due to a postoperative hematoma, and once due to a postoperative abscess)."

Comment 5: *Might be worth mentioning another limitation is the follow-up period for complications. This was limited to POD3 while in hospital and some bile duct injuries or other delayed injuries could present later.*
Reply 5: We completely agree with you. The very short follow-up period is a limitation of the study. We have mentioned this in the discussion section.
Changes in the text: Discussion: "Limitations of our study are the monocentric design, the small patient cohort and the limited follow-up period of only three postoperative days, which should be considered when interpreting the results."

REVIEWER C
Comment 1: *"It is mentioned the majority of the LiVac cases were performed by residents when compared to the Gan 3 port study. Was there a learning curve evident with repeat use? The significantly increased time may be exaggerated by lack of familiarity to the device not just increased difficulty."*
Reply 1: You are absolutely right in your comment. Of course, the lack of familiarity with the retractor might also have an impact on the operation time. However, it was rather our impression that the operation was prolonged by the poor visualization of the surgical field. Unfortunately, we cannot identify the exact reason from our data.
Changes in the text: Discussion: "Thus, the longer operating times and poor satisfaction with the LiVac retractor in our study may also reflect a lack of familiarity of surgeons with the device. The possible effect of a learning curve was not investigated in our series."

Comment 2: *Line 239 "It should also be emphasized that LiVac application...". This statement is out of place compared to the beginning of the paragraph. May need to include more of a transition statement or to break the thought into a separate paragraph.*
Reply 2: Right, thank you very much. We have revised the passage.
Changes in the text: Discussion: "…There were no benefits found for patients in the LiVac group in terms of postoperative pain levels, as differences in NRS were statistically not significant.
Despite the mentioned problems with LiVac, it should be also emphasized that its application did not result in sonographically visualizable subcapsular liver hematomas, nor did it cause any remarkable changes in laboratory values.
In contrast to our results, Chiung and Gan found reduced postoperative opioid need…"

REVIEWER D
Comment 1: "*Also there was a astonishing high proportion of the patients in the Livac group that crossed over to the control group-or what happened actually to this 60% (the majority) where the Livac was abandoned. These were probably the most difficult cholecytectomies and still they had shorter optimes and fewer complications.*"
Reply 1: We have to apologize as we did not describe the evaluation sufficiently. The number of LiVac patients remained unchanged because even after switching to laparoscopic 4-port cholecystectomy, they didn´t change to the other group, nor were they excluded. The reason for this was to avoid an attrition bias. We tried to clarifiy this in the Methods section.
Changes in the text: Methods: "Groups were analyzed per protocol. If the application of the LiVac retractor was unsuccessful in patients of group 1, the procedure was switched to the standard 4-port technique. To avoid attrition bias, these patients were not excluded, but still evaluated as LiVac patients."

Comment 2: *"The study is also to small and not randomized making the results hard to generalize."*
Reply 2: This statement cannot be contradicted, you are absolutely right. The limitation of a small study group is also mentioned in the discussion section.
Changes in the text: Discussion: "Limitations of our study are the monocentric design, the small patient cohort and the limited follow-up period of only three postoperative days, which should be considered when interpreting the results."

REVIEWER E
Comment 1: *"Although this is not a randomised controlled study, the prolonged operating time, 10% associated morbidity, and bile duct injury from this system make it clear that it is unsafe and must not be used. However, the conclusions do not reflect their experience, and the authors mentioned that it is safe. Although the study is negative, it gives at least some evidence supporting the fact that LiVac has very little role to play during cholecystectomy.*"
Reply 1: We completely agree with you. We had probably formulated our opinion too cautiously. The passage has been revised.
Changes in the text: "It can be concluded that laparoscopic 3-port cholecystectomy with the LiVac retractor is feasible. However, we think that LiVac should only be

used by a) experienced laparoscopic surgeons, that b) are very familiar with the device, and c) when standards such as the "Critical View of Safety" are strictly followed. Due to the specifics regarding handling and limited exposure of the operative field with the LiVac system mentioned above, 3-port LC is hardly suitable as a training procedure, and is not appropriate for wide application."

Statistical test-assumptions (REVIEWER A)

The requirements that the tests used in this paper must meet were taken from the following book:
Verma, J. P., and Abdel-Salam G. Abdel-Salam. *Testing Statistical Assumptions in Research*, John Wiley & Sons, Incorporated, 2019. *ProQuest Ebook Central*.
Chi-square test

The chi-square test is a non-parametric test with three prerequisites:

1. *"There must be two or more categories for each of the two categorical variables.*
2. *The observations in each group should be independent and expected frequencies for each category should appear only once. Both the chi-square test for goodness and the test of independence are not appropriate for paired samples.*
3. *The samples size should be large enough to be ensure that the expected frequency in each cell is at least 1, and the majority of cell have the expected count of at least 5. "*

(S. 148 Verma, J. P., and Abdel-Salam G. Abdel-Salam. *Testing Statistical Assumptions in Research*, John Wiley & Sons, Incorporated, 2019. *ProQuest Ebook Central*.)

The first point is given, the two variables whose relationship is to be studied with this test meet the criteria. Both the group membership (LiVac vs. control) and the complication variable are categorical variables. They have two or more characteristic values. Thus, this assumption is fulfilled.

The second assumption does not require paired data, which is not present here because no pairs can be formed between the two groups (LiVac vs. control), also the control group is significantly larger. Further, the categories should be independent of each other. (At this point I would recommend you to briefly explain to the reviewers why your selection process guarantees this). Thus, the second assumption is also given.

For the last assumption, the number of cases must be large enough for each field to be occupied, and further, there must be at least 5 features in 80% of the fields. Since there are fortunately so few complications, I would advise you to combine the complication variable into "complications" and "no complications". This condition would be fulfilled. Now the frequency distribution looks like this:

LiVac Group

Clavien Dindo Classification	No Complications	1	3
Quantity	21	3	1

Control Group

Clavien Dindo Classification	No Complications	1	2	3
Quantity	44	2	1	2

Mann-Whitney-U-Test

Like the chi-square test, the Mann-Whitney-U test is a non-parametric test. It also makes three assumptions:

1. *"The data must be obtained from two independent random samples; there should be two independent categories of the independent variable to test the group differences.*
2. *The test aims to compare the difference between two distributions of the random samples. The shape (variability) of the distribution is assumed to be the same, and only the location (central tendency) is allowed to vary across the groups.*
3. *The dependent variable can be either ordinal or continuous but not normally distributed."*

(S. 157 Verma, J. P., and Abdel-Salam G. Abdel-Salam. *Testing Statistical Assumptions in Research*, John Wiley & Sons, Incorporated, 2019. *ProQuest Ebook*.)

The first assumption is given by the research design.

The second assumption, similar distributions are now tested for each variable in turn by comparing mean, variance, skewness and kurtosis. The mean values do not have to be similar; they only serve as an indication for better interpretation of all the other parameters.

#NRS1
Mean LiVac: 2.06
Mean Control: 1.541667
Variance Livac: 4.048333
Variance Control: 1.73227
Skewnes LiVac: 1.281089
Skewnes Control: 0.3826042
Kurtosis LiVac: 4.487051
Kurtosis Control: 2.215938
The distributions of these two variables differ noticeably from each other, so it is quite possible to speak of a violation of the prerequisites.

#NRS3
Mean LiVac: 0.42
Mean Control: 0.7083333
Variance Livac: 0.6808333
Variance Control: 1.019504
Skewnes LiVac: 2.019991
Skewnes Control: 1.250506
Kurtosis LiVac: 6.112978
Kurtosis Control: 3.455555
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#AST0
Mean LiVac: 27.64
Mean Control: 26.75
Variance Livac: 405.0733
Variance Control: 423.2979
Skewnes LiVac: 3.828467
Skewnes Control: 4.063638
Kurtosis LiVac: 17.6786
Kurtosis Control: 20.01765
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#ALT0
Mean LiVac: 31.84
Mean Control: 43.02083
Variance Livac: 421.4733
Variance Control: 4216.106
Skewnes LiVac: 2.124942
Skewnes Control: 4.444691
Kurtosis LiVac: 7.700518
Kurtosis Control: 24.96205
The distributions of these two variables differ noticeably from each other, so it is quite possible to speak of a violation of the prerequisites.

#GGT0
Mean LiVac: 97.36
Mean Control: 72.75
Variance Livac: 17656.49
Variance Control: 4649.894
Skewnes LiVac: 2.088012
Skewnes Control: 1.725906
Kurtosis LiVac: 6.104798
Kurtosis Control: 5.471444
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#ALP0
Mean LiVac: 85.16
Mean Control: 85.1875
Variance Livac: 2105.557
Variance Control: 1193.347
Skewnes LiVac: 2.539166
Skewnes Control: 1.626314
Kurtosis LiVac: 10.12958
Kurtosis Control: 6.759408
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#CRP0
Mean LiVac: 0.632
Mean Control: 0.3468085
Variance Livac: 3.3806
Variance Control: 0.1381961
Skewnes LiVac: 4.445107
Skewnes Control: 1.812069
Kurtosis LiVac: 21.44739
Kurtosis Control: 5.469479
The distributions of these two variables differ noticeably from each other, so it is quite possible to speak of a violation of the prerequisites.

#WBC0
Mean LiVac: 6.816
Mean Control: 6.73125
Variance Livac: 3.117233
Variance Control: 3.83879
Skewnes LiVac: 0.4094646
Skewnes Control: 1.029647
Kurtosis LiVac: 2.801821
Kurtosis Control: 4.555069
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#AST1
Mean LiVac: 70.35294
Mean Control: 67.2381
Variance Livac: 598.8676
Variance Control: 760.8905
Skewnes LiVac: 0.5647494
Skewnes Control: 1.078113
Kurtosis LiVac: 2.961112
Kurtosis Control: 4.094443
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#ALT1

Mean LiVac: 95.88235
Mean Control: 81.66667
Variance Livac: 2249.86
Variance Control: 2274.233
Skewnes LiVac: 0.6085851
Skewnes Control: 1.451341
Kurtosis LiVac: 2.684036
Kurtosis Control: 4.85326
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#GGT1
Mean LiVac: 76.76471
Mean Control: 72.85714
Variance Livac: 5408.691
Variance Control: 3425.329
Skewnes LiVac: 1.51675
Skewnes Control: 1.386707
Kurtosis LiVac: 4.014089
Kurtosis Control: 4.531048
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#ALP1
Mean LiVac: 81.70588
Mean Control: 77.38095
Variance Livac: 2877.971
Variance Control: 1063.548
Skewnes LiVac: 2.673116
Skewnes Control: 1.064353
Kurtosis LiVac: 9.851383
Kurtosis Control: 3.424327
The distributions of these two variables differ noticeably from each other, so it is quite possible to speak of a violation of the prerequisites.

#CRP1
Mean LiVac: 5.694118
Mean Control: 4.056522
Variance Livac: 62.93684
Variance Control: 26.4653
Skewnes LiVac: 1.907643
Skewnes Control: 1.496061
Kurtosis LiVac: 5.524733
Kurtosis Control: 3.944015
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#WBC1
Mean LiVac: 9.170588

Mean Control: 9.313043
Variance Livac: 11.57096
Variance Control: 7.364822
Skewnes LiVac: 1.789278
Skewnes Control: 0.000220437
Kurtosis LiVac: 4.954164
Kurtosis Control: 2.413813
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#AST3
Mean LiVac: 60
Mean Control: 37.375
Variance Livac: 6810.5
Variance Control: 245.7258
Skewnes LiVac: 3.107001
Skewnes Control: 1.849206
Kurtosis LiVac: 10.82456
Kurtosis Control: 7.328121
The distributions of these two variables differ noticeably from each other, so it is quite possible to speak of a violation of the prerequisites.

#ALT3
Mean LiVac: 100.7692
Mean Control: 65.53125
Variance Livac: 16539.19
Variance Control: 1287.934
Skewnes LiVac: 2.9502
Skewnes Control: 1.452556
Kurtosis LiVac: 10.22312
Kurtosis Control: 5.897471
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#GGT3
Mean LiVac: 125.7692
Mean Control: 88.875
Variance Livac: 6596.026
Variance Control: 5780.306
Skewnes LiVac: 0.8019669
Skewnes Control: 1.552898
Kurtosis LiVac: 3.427958
Kurtosis Control: 4.9258
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#ALP3
Mean LiVac: 94.07692
Mean Control: 82.34375

Variance Livac: 2847.91
Variance Control: 963.5877
Skewnes LiVac: 1.779367
Skewnes Control: 1.641169
Kurtosis LiVac: 5.682275
Kurtosis Control: 7.229243
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#CRP3
Mean LiVac: 5.784615
Mean Control: 7.721875
Variance Livac: 62.13974
Variance Control: 97.26564
Skewnes LiVac: 2.025359
Skewnes Control: 1.555458
Kurtosis LiVac: 6.212367
Kurtosis Control: 4.259043
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

#WBC3
Mean LiVac: 7.407143
Mean Control: 6.684375
Variance Livac: 5.986868
Variance Control: 3.783942
Skewnes LiVac: 0.8542343
Skewnes Control: 0.9698775
Kurtosis LiVac: 2.45239
Kurtosis Control: 3.463236
All in all, these are similar distributions, so for this variable this assumption can be taken as given.

The distributions of the variables of both groups were compared and in most cases the variables were similar, so the second condition was fulfilled. In five cases there were deviations between the distributions: NRS1, ALT0, CRP0, ALP1, AST3.

For the third assumption the dependent variables must not be normally distributed. In order to check for normality, the Shapiro-Wilk normality test was performed.

The Shapiro-Walk test determined a result significant at the 90% significance level for all variables except the following:

GOT2 (control), AST1 (LiVac), WBC0 (LiVac), GGT3 (Livac), WBC4 (control), WBC4 (LiVac).

For this reason, all other variables are to be considered as non-normally distributed.
A closer look at the skewness and kurtosis of all other distributions where the test does not work shows that they do not quite correspond to the normal distribution.
Thus, the third condition is also fulfilled.

The dependent variables have scale.

Conclusion

In conclusion, in most cases all assumptions are met and only in a few exceptions a single assumption is not completely true, so special care should be taken when interpreting the results of these variables.
