Author’s response to reviews

Title: Staphylococcus aureus colonization in hemodialysis patients: a prospective 25 months observational study

Authors:
Matthias Scheuch (matthias.scheuch@uni-greifswald.de)
Sabrina Freiin von Rheinbaben (sabrina.freiinvonrheinbaben@uni-greifswald.de)
Antje Kabisch (antje.kabisch@geo.tu-darmstadt.de)
Jonas Engeßer (je124094@uni-greifswald.de)
Susanne Ahrendt (susanne.ahrendt@kfh-dialyse.de)
Thomas Dabers (thomas.dabers@uni-greifswald.de)
Christian Kohler (christian.kohler@uni-greifswald.de)
Silva Holtfreter (silva.holtfreter@uni-greifswald.de)
Barbara Bröker (broeker@uni-greifswald.de)
Sylvia Stracke (sylvia.stracke@uni-greifswald.de)

Version: 1 Date: 21 Feb 2019

Author’s response to reviews:

Dear Dr. Meijers,

we appreciate your interest in our work and thank you and the reviewers for your constructive criticism of our manuscript. We changed the manuscript according to your and the reviewers’ suggestions thus improving our work.

The most important changes include (1) the removal of the renal healthy control cohort due to the small sample size and (2) the standardization of the study duration to 25 months.

Table 2 has now been added to the supplemental data due to its size. This table contained our raw data about carrier state, colonization type and vascular access of each individual patient. Summaries to these data are presented in the manuscript in the results section ‘S. aureus colonization and carrier status’ and ‘Characterization of the S. aureus strains and comparison with general population’, in the discussion section ‘Low S. aureus prevalence in SaDial study on hemodialysis patients’ and ‘Different S. aureus properties in hemodialysis patients’ as well as in figures 1 and 3 and in table 2 (formerly table 3).
Below, there is a point-by-point response to all of your comments and questions. Changes are indicated by the line information. For all changes in the manuscript, we used the Microsoft Word track changes. All figures have also been revised for a better resolution.

Our main findings remained unchanged: compared to the general population, hemodialysis patients were more frequently colonized with S. aureus and displayed both different S. aureus colonization densities as well as lineages, possibly explained by more frequent exposure to health care environments.

Yours sincerely,

Dr. Matthias Scheuch
Prof. Dr. Sylvia Stracke

Wilbert Van Der meijden (Reviewer 1):
A nice and fascinating study. I have some questions and comments:

Minor points
Which cannulation technique did you use: buttonhole or rope ladder cannulation? We are using rope ladder cannulation for dialysis to avoid aneurysms.

Did you see difference in carriage, amount of colony forming units or infections with S. aureus? We solely use rope ladder cannulation so we cannot compare the rates with buttonhole cannulation.

The main causes of death in hemodialysis patient in these study population were bloodstream infections by pathogens other than S. aureus. How much were caused by proven line infections?

Only one patient died from sepsis due to a proven line infection caused by Klebsiella oxytoca. Further five patients had a suspected line infection, but no pathogen could be detected. In total, 16 patients died from sepsis. In 11 cases, the pathogen could be found. We changed table 4 accordingly and now show the causes of death and the related pathogens more clearly. Furthermore, we added the corresponding information to lines 316-319 to the manuscript.

Did you also investigate the influence of treatment with antibiotics (for other reasons) and carrier ship?

During the investigation time of the study, no unusual antibiotic resistances occurred neither in hospital nor in the outpatient dialysis center, which would have given rise to the investigation of possible resistance developments in patients.

S. aureus strains genetically analyzed with the DNA-Microarray were also investigated for the
presence of resistance genes, but we did not find any resistance patterns. A precise analysis with regard to antibiotic use in individual S. aureus carriers and the presence of resistance genes in strains isolated from carriers was not performed. Neither did we investigate the influence of antibiotic treatment with respect to carriership.

However, this is an interesting approach that we may take up again later.

Twenty (you mention 10 in the abstract) surgical patients were recruited as controls free from renal disease. This is not a real healthy control group. Do you have data about re-hospitalization, visiting the outpatient clinic, need antibiotics etc.?

10 in the abstract is a mistake, 20 would be correct. Renal healthy control patients were recruited at the beginning of the study with the aim of making a comparison between patients with chronic kidney failure, renal healthy patients but with contact to medical staff and a healthy general population. The question was if renal healthy patients compared to those with chronic kidney failure already harbor different S. aureus strains and if the proportion of carriers has already changed due to the hospitalization but not due to the frequent contact to dialysis staff. We recorded data on patient characteristics and medical histories similar to the hemodialysis population to ensure these patients were not in contact to an outpatient dialysis center. Unfortunately, this renal healthy control cohort is too small for a comparison or any statistical evaluation so it will not be considered any longer and we will only take data from the general population into account. According to the reviewer’s and the editor’s recommendation, we removed this small cohort from the manuscript (lines 41, 131, 218 – 219, 229 – 230, 235 – 236, 259 – 261, 616 – 617, 620 and 623 – 624) and the figures 1 and 2.

Line 116: You mention three years observational study: it is two years
The entire study ran over 3 years. The actual sampling of patients conducted from 02/2016-02/2018 which is exactly 25 months but the study started earlier with recruitments, preparations etc. We decided to take 25 months for time in the whole manuscript and adjusted accordingly the title and the lines 37, 43, 118, 292, 308, 326, 335 and 413 as well as the X axis in figure 5.

Amaryllis Van Craenenbroeck (Reviewer 2):
The study by Scheuch et al addresses the relevant topic of S. aureus carrier state in patients on hemodialysis. They show that the prevalence of S. aureus carriers was higher in hemodialysis patients compared to controls. When studied longitudinally, the carrier state even increased to 65% (total of intermittent and persistent carriers). These findings certainly prompt to further research. The authors conclude also on mortality.

Major points
In the Cox regression analysis, no attention was paid to competing event(s). An alternative method for analysing competing risks data should be applied before conclusions can be drawn. The cox regression analysis revealed age and carrier status as factors influencing overall mortality in our hemodialysis population significantly.
We also took other factors from the patient questionnaire as possible confounders or competing risks (age, duration of dialysis (years), gender (male/female), dialysis access (CVC/ AV fistula), S. aureus carrier status (carrier/non-carrier), BMI, diabetes status (diabetic/non diabetic), presence of foreign body material (CVC, implants), previous ICU stays, living situation (home/rest home, assisted living), (former) alcohol consumption, smoking behavior (smokers, ex-smokers/non-smokers) and previous bloodstream infection) into account. This way, we considered only the group of carriers, added one factor after another (cumulated and not cumulated) and calculated changes within the model coefficients. Except for age (12.5%), for none of the factors the coefficient changes for more than 8% indicating only age as a weak confounder for overall mortality in S. aureus carries.

Because the number of influencing factors is limited by the number of events (deaths in our case) in the cox regression model, it is theoretically not permitted to calculate for more than 3 factors in our case. Therefore we also used a multiple logistic regression model in parallel. Within this model we may take all factors into account but were not able to consider the time of death any longer. The logistic regression conditions of no outliers, no multicollinearity and linearity of logits were tested previously and fulfilled. Also this model revealed age and carrierrship as factors with significant impact on overall mortality; plus a previous sepsis as a further factor. Similarly to the cox regression model, we also took the same confounders as above for overall mortality into account, revealing again only age (34%) as a very strong influencing factor on the subpopulation of S. aureus carriers (<9% for the others).

Because the cox regression model is more appropriate in a survival analysis, we have decided to use this method. However, we will amend the corresponding passage (lines 199 – 202) in the manuscript with the necessary information for competing risks.

The time flow of the article is very hard to follow. Information on the length of the study is misleading in the title/abstract. Is it 3 years (title), 24 months (introduction) or 2.5 years (methods)? From the Methods’ section of the Ms, the study seems to be conducted form 02/2016-02/2018 (2 years) with 5 measurements.

The duration of the study is 3 years. The actual sampling of patients conducted from 02/2016-02/2018 which is exactly 25 months but the study started earlier with recruitments, preparations etc. We decided to take 25 months for time in the whole manuscript and adjusted accordingly the title and the lines 37, 43, 118, 292, 308, 326, 335 and 413 as well as the X axis in figure 5.

Demographic data on the controls is lacking. Moreover, the authors chose a rather small control population. What could be the advantage of such a control population? Why not comparing the findings with studies with a larger number, such as the SHIP-TREND-0? The rationale is hard to find in the Ms; please add a comment in the introduction.

We also recorded data on patient characteristics and medical histories of the renal healthy control similar to the hemodialysis population to ensure these patients were not in contact to an outpatient dialysis center. Unfortunately, this renal healthy control cohort is too small for a comparison or any statistical evaluation so it will no longer be considered and we will only take data from the general population (SHIP-TREND-0) into account. According to the reviewer’s
and the editor’s recommendation, we removed this small cohort from the manuscript (lines 41, 131, 218 – 219, 229 – 230, 235 – 236, 259 – 261, 616 – 617, 620 and 623 – 624) and the figures 1 and 2.

The number of patients/controls studied is not clear. In the abstract the authors mention 10 controls, in the Methods' section, they say that they included 20 surgical patients. 10 in the abstract is a mistake, 20 would be correct. However, according to the editor’s recommendation, we removed this small cohort from the manuscript (lines 41, 131, 218 – 219, 229 – 230, 235 – 236, 259 – 261, 616 – 617, 620 and 623 – 624) and the figures 1 and 2.

The results' section of the Ms contains several interpretations, which is more suitable for discussion. Suggestion to stick to the data in this section.

We have reviewed the results’ section and revised it according to the reviewer’s suggestion. We removed two passages that were either redundant or were already covered in the discussion part:

1. We removed the interpreting sentence “The fact that samples were taken from different patients with different colonization patterns and at different time points also argues against an outbreak situation at the dialysis center.” in lines 265-267.

2. Lines 277 – 289 were removed from the results’ section. We had discussed this already in the discussion (lines 387-395).

Table 2 is too extensive. The data should be summarized in a concise way, for example number/strain etc (cfr Table 3). It contains a lot of information, but the main message is not clear. It could be added as Supplemental Data?

We will add table 2 as supplemental data.

Table 5 has the same problem. The reader is not interested in the time of death; these are represented in the survival analysis.

We skipped the column with ‘Time of death’. The column ‘Cause of death’ is now divided into ‘Cause of death’ and ‘Pathogen detection’.

The text in the Figures is illegible (Fig 1-3)
The resolution of figures looks completely different to what we uploaded at the submission. We adjusted it.

Minor points

what is a 'renal healthy control' (abstract)? This expression is subject to interpretation.

Renal healthy control patients were recruited at the beginning of the study with the aim to make a comparison between patients with chronic kidney failure, renal healthy patients but with contact to medical staff and a healthy general population. The question was if renal healthy
patients compared to those with chronic kidney failure already harbor different S. aureus strains and if the proportion of carriers has already changed due to the hospitalization but not due to the frequent contact to dialysis staff. Because of the small amount of data we will now just focus on the comparison between our hemodialysis study population and the general population from SHIP-TREND-0.

P3, line 73. Do the authors mean immunocompromised?
Yes, we changed it to immunocompromised.

P5, line 101. Can you give a reliable reference for the statement that in (only) about 15-20% of cases, a permanent CVC is used? In their study population, the use appears to be 33%.

This is actually a mistake based on a physician statement. We will adjust the percentages according to Astor et al., 2005 to 34% for CVC use after 6 months. The percentage is high but attributed to the fact that incident hemodialysis patients are often elderly patients and start treatment with a CVC instead having a fistula created in time. The new reference will be added to the manuscript under the reference number [9].


How did the authors reveal the cause of death? This should at least be mentioned in the Methods' section.

The causes of death were taken from the final medical reports in the outpatient dialysis center. Four of the authors (S.R., S.A., T.D. and S.S.) are the nephrologists in charge for the patients reported here. All information contained in the medical charts were evaluated again and summarized for the study. We added a corresponding section into the methods part (lines 136 – 139).

Editor comments:
The findings in hd patients are of interest, but deserve better characterization.

Please express rates of carrier state and infection also/ months of access type (fistula vs. Catheter).

In total, we had 1,147 months in which patients were dialyzed via arteriovenous fistula and 359 months with a central venous catheter as a dialysis access. With 2 S. aureus infections in patients with an AVF access it corresponds to a rate of 0.002 S. aureus infections per month, 0.01 for CVC patients (4 S. aureus infections in 359 months) respectively. These data were added to the ‘S. aureus infections’ part within the results section (lines 299 – 300). Unfortunately, we have no data corresponding to the global infection rates apart from S. aureus because those cases were not recorded.
Within the 1,147 AVF months, there were 445 months in which patients were colonized with S. aureus corresponding to a carrier rate of 0.39 per month, 0.46 for CVC months (165 months with S. aureus carrier in 359 CVC months) respectively. These data were added to the ‘S. aureus colonization and carrier status’ part within the results section (lines 219 – 220).

The contribution of healthy controls to the current study is questionable, predominantly due to small sample size. Please expand the number of healthy controls or remove altogether.

The recruitment of further control patients is very difficult, in particular when the sample size has to be increased in a manner to allow statistical comparisons to the dialysis cohort. So we decided not to consider our renal healthy control cohort for longer and focus only on general population for comparisons. The corresponding parts regarding the renal healthy control cohort in the manuscript (lines 41, 131, 218 – 219, 229 – 230, 235 – 236, 259 – 261, 616 – 617, 620 and 623 – 624) and in figure 1 and 2 were removed.