Reviewer's report

Title: Molecular epidemiology of a hepatitis C virus epidemic in a haemodialysis unit: outbreak investigation and infection outcome.

Version: 2 Date: 26 April 2010

Reviewer: Nicola D Thompson

Reviewer's report:

1) In the second sentence of the background, the authors imply in developed countries the highest prevalence of HCV infection is among “...subject who are frequently exposed to invasive medical procedures, such as hemodialysis patients. Is this correct? This is not the case in the UK or the US. In what countries? Please provide appropriate references for this statement. If the intent is to state that patients who undergo hemodialysis treatment have an increased prevalence of HCV infection, please change this sentence to reflect that, or clarify what is meant by “invasive medical procedures”.

2) The authors provide case definitions used for the investigation, but it is unclear from your methods or the results sections how the 13 patients reported to have new HCV infection were identified. What triggered the indentified of this outbreak? Were all of 50 patients in the cohort were tested? A non-case (of HCV susceptible) definition is not used; it would be helpful for case accounting purposes to have one. How were those without HCV infection (e.g. the non-cases) determined? What HCV test was used? While there is currently a section in the methods called Virologic methods, which includes one sentence on anti-HCV testing. It would be helpful to either: add a short section to the methods to describe the serologic testing that was performed and who was tested (all patients, all staff?) and when, or to change the title and current information in the Virologic methods to include the serology testing methods. Were all patients tested for the presence of HCV RNA? What about genotype, and molecular analysis. At each stage of testing describe who/what was tested?

3) In order to help the reader understand what patients or sub-set of patients are being referred to, please use the case definitions consistently throughout the manuscript. For example, newly infected patients, incident cases and became anti-HCV positive are all used to describe the group who had newly acquired HCV infection. The term susceptible patients is used, but is not defined in the methods section. Prevalent and chronically infected patients (molecular analysis results paragraph), and chronic HCV carrier (discussion).

4) The first paragraph under auditing procedure is a description of what you did. This paragraph belongs in the methods section, not the results.

5) In the methods it states a retrospective cohort study is performed, yet in the results you report odds ratios (OR) as the measure of association. The
appropriate measure for a cohort study is the Risk Ratio, please can you clarify why odds ratios are reported?

6) Statistical methods: The description of the analysis in the methods includes an assessment of risk factors that include being dialyzed after or along a HCV gen-2 positive subject. What is the relevance of this? This appears to be the use of information that should be in the results section in the methods section. Please re-write this so that results are not used as part of the methods.

7) Results. Please move the section called “clinical setting and population at risk” to the beginning of the results section, including the data presented in Table 1. Also, the first paragraph under clinical follow-up on acute HCV infection would also be more informative to the reader if it were included in the first paragraph or two of the results section.

8) Statistical methods and results: The description of the analysis in the methods includes an assessment of risk factors that include being dialyzed after or along a HCV gen-2 positive subject. However, in the 2nd paragraph of the methods section it states, “As reported in table 2, unadjusted analysis evidenced that having been dialyzed at least once either after (p=0.035; OR= 5.50 [1.05-28.88]) or in the same session (p=0.029; OR= 0.21 [0.05-0.89]) of a HCV positive subject …”. In the results, do you mean any HCV positive subject or should this be the HCV gen-2 positive subject, as stated in the methods? Please clarify how these variables were constructed and the data analyzed.

9) The statistical analysis appears to include all 13 patients with new HCV infection; however, HCV characterization was completed only for 10 or 13, and analysis by HVR1 only for 8 or 13. Here, the assumption is made that the single HCV genotype 2c prevalent case was the source of HCV infection also for those that could not undergo characterization. Do the authors think the 3 patients without HCV characterization are also associated with the same HCV genotype 2c prevalent case? Were these 3 patient seen on April 20th? What epidemiologic data supports that the HVC genotype 2c prevalent case was the likely source of their infection? How common/rare is genotype 2c in Italy and/or this regions? Could other prevalent cases (i.e. the 3 that were not available) have been the source of their infection? Please address this in the discussion (limitations?) section, and the potential for introducing exposure misclassification into the statistical analysis.

10) General organization : It would improve the flow and comprehension of the paper if the order and headings used in methods and results sections mirrored one another. As currently written, the methods section is very difficult to follow and confusing. For example, the case definitions make use of methods information that has not yet been presented (the virologic methods).

a. The 33 weeks retrospective analysis – is this the definition of the study period (when you believe transmission most likely occurred? If yes, please state that and why you selected that period. Is this the period used for the data analysis described in the statistical analysis described later on in the methods section? If
so, it would be helpful to have these two pieces of information together.
b. 24 weeks prospective surveillance. Prospective surveillance for what? Are the results of the prospective surveillance reported in the results? Clarify what this means and the relevant findings in the results section.
c. The order of the final 3 bullets does not make chronological sense. Suggest placing auditing procedures first, then molecular characterization, and finally 1-year follow-up.
d. the section called “Clinical setting and population at risk” is a description of the hemodialysis unit and general operations, and the number of sessions performed during the study period and refers to Table 1 – a description of patients in the facility. By all accounts, this information appears to be results, and would thus be better suited to the results section.

11) What was the size of the multi-dose heparin vial? Please report this information. How many heparin doses (2ml) were in each vial? Could one heparin vial have been the source for all 11 patients seen in April 20th? In what format/size was the saline that was drawn into a syringe with the heparin? What was format (single dose, multi-dose, or saline bag), and what size was the saline? How was the saline used (for one patient and then discarded, or for multiple patient). Have you considered the possibility that the saline became contaminated and was the vehicle of HCV transmission? See: Macedo de Oliveira, et al. An outbreak of hepatitis C virus infections among outpatients at a hematology/oncology clinic. Ann Intern Med. 2005;142:898-902.

12) Figures 2 and 3 need a descriptive figure legend. For figure 3, what do the percentages at the top of each bar refer to (the % of sessions with a HCV positive patient attending), this should be made clear to the reader. For figure 3, 3 prevalent cases were not genotyped. It would provide a better overall picture of the outbreak if this figure also included the session when these three cases also attended. Could be shown as “HCV positive – unknown genotype”.

13) Figure 4 requires a descriptive figure legend and key, and additional information to orient the reader to the cases and interpretation.

14) Table 3: the data presented in table 3 is all reported in the manuscript text, and is therefore redundant. Suggest deleting the table, and ensuring all of the content is reported in the text in the appropriate section of the results.

Minor Essential Revisions:

1) Delete final sentence at the end of the first paragraph of the abstract as it duplicates the prior sentence.

2) Throughout the manuscript a number of different terms are used for HCV antibody (for example; anti-HCV in the abstract, anti-HCV-Ab in the 2nd paragraph of the background, HCV-Ab in the 1st paragraph of the Epi and Clin definitions, anti-HCV antibody in the 1st paragraph under virological methods). Please standardize the used of this term throughout the manuscript. The
standard is to use “HCV antibody (anti-HCV)” for its first use, and then to use “anti-HCV” thereafter.

3) Under molecular analysis results, please include the HCV genotype for the second HCV prevalent case that underwent genotyping?

Discretionary Revisions

1) Figures 2 and 3 are not very informative in their current format and do not add substantially to manuscript. Consider, a) re-working the format so the information is more meaningful to the reader, b) deleting these figures from the manuscript and providing a short explanation in the text of the results.

2) Results. In the first paragraph under clinical follow-up: An increase in ALT is a sign (physical manifestation of illness and not subjective illness experienced by the patient), not a symptom of acute HCV infection. Suggest a change from symptom to sign.

3) Suggest an outline similar to the one below for the order of the methods, and for the reporting result:

i. Clinical setting and population at risk (report what your methods not results)
ii. Serologic/virologic methods and molecular characterization
iii. Case definitions (including non-case)
iv. Epidemiological definitions (Prevalence rate/Attack rate/clinical AR)
v. Retrospective cohort analysis and statistical methods
vi. Auditing procedures
vii. 1 year clinical follow-up and clinical outcome definition.

Level of interest: An article of importance in its field

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

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests