PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Collecting biological material from palliative care patients in the last weeks of life: a feasibility study
AUTHORS	Coyle, Seamus; Scott, Aileen; Nwosu, Amara; Latten, Richard; Wilson, James; Mayland, Catriona; Mason, Stephen; Probert, Chris; Ellershaw, John

VERSION 1 - REVIEW

REVIEWER	Prof Rob George St Christopher's and Cicely Saunders Institute KCL
REVIEW RETURNED	04-Apr-2016

GENERAL COMMENTS	My comments are on style, not content. This is a good read and important, hence my concern that it is punchy and crisp UK english as I hope it is referenced often as a method. There are too many passive tenses and split infinities - make for clumsy sentencing and many more words than necessary. I've given the first few examples in the attached file. There are more. The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

REVIEWER	Janet Hardy Mater Health services Brisbane, Australia Prof Hardy sits on the medical advisory boards of Mundipharma and Menarini Aust Pty Ltd. Any honoraria are directed to charity.
REVIEW RETURNED	30-May-2016

GENERAL COMMENTS	This paper raises a number of interesting moral and ethical issues and is a very good example of a novel consent process but fails to prove that collecting biological material from dying patients is feasible. As highlighted below, only about 10% of all potential participants provided urine samples in the last weeks of life (if this is defined as in the month prior to death). Specific comments: -Title. This is deceptive. This is not a paper describing the collection of biological material in the last weeks of life but a paper about the collection of urine samples from patients with advanced disease. -Summary. Minor point; typo in last sentence of strengths and limitations -Introduction. What work if any has been done to date on the value of studying urine samples during the dying process. What specific molecular or metabolomics approach might provide useful

	information? -Methods. Were research staff employed specifically to carry out this project or was it the unit staff collecting the samples? -the paper alludes to the difficulty of gatekeeping in conducting research in palliative care on several occasions and yet this is an example of gate keeping at the extreme. Senior medical staff had to screen and make a decision as to whether any potential participant could be approached. This effectively prevented a number of patients from making up their own mind about whether or not they would like to participate. This deserves further discussion especially in light of previous research in this area. -minor point, why was smoking status relevant in the collection of demographic date? - the authors have addressed the issue or not over-burdening participants with lengthy information sheets to some extent in the discussion. It would be interesting to know more about what was in the 3 page patient information sheet. What were participants told about what their urine might be tested for? Could the PICF be included as an appendix? -by agreeing to donate urine for some future molecular or metabolomics research, participants were actually contributing to a form of tissue bank. This raises a number of other ethical issues. Did the participants consent to their urine being used for any non-specified test that might come along in the future? Might this involve genetic testing in which case a separate consent form is often required. Once again, it would be valuable to include more about the information given to participants -I congratulate the authors on the development of the consultee declaration process but query why this had to be repeated every time a urine sample was taken. This seems an unnecessary barrier to place around each collection. Investigators could determine if a participant was not well enough to make a further donation. I would suggest that this issue of consent is the strongest aspect of the paper and could be the focus -the greatest weakness of this study must be that only one-half the participants provided samples in the last 4 weeks of life and that almost one-third were alive more than 3 months after the study. In fact, only 11 out of 103 in-patients provided samples in the month prior to death. As alluded to early, it cannot be said that this study proved the feasibility study of collecting samples from dying patients. -the information in table 2 could be presented in the text, but the time to death information is crucial -Discussion. This paper implies that palliative care patients form a particularly vulnerable population that deserve special protection from the rigors of scientific research. I would suggest that this population is no more vulnerable than many other groups (for example neonates and those in intensive care), who are commonly offered trial participation in order to find the evidence to improve the care of others. Moreover, there is now extensive literature showing how patients at the end of life are very willing to be involved in research especially that which is non-invasive (eg White C et al, Randomized controlled trials of palliative care - a survey of the views of advanced cancer patients and their relatives. Eur J Cancer (2008); 44: 1820-1828). These points could be discussed further. Conclusion. It is stated that the protocol used minimised stress to patients and their families. It may be worth highlighting the increasing number of clinical studies that have been completed in this population group over recent years, none of which have identified any major patient or carer concerns. Moreover, many of these have been randomised controlled studies involving interventions

	considerably more invasive than this one eg those from the Australian palliative care research Collaborative PaCCSC (http://www.caresearch.com.au/CareSearch/tabid/2476/Default.aspx)

REVIEWER	Nicholas Wysham Duke University, Durham, NC, USA
REVIEW RETURNED	27-Jun-2016

GENERAL COMMENTS	Introduction The authors present a feasibility study of collecting urine samples from the terminally ill in a hospice unit. While a reader who does not conduct research in hospice units might initially underestimate the multiple challenges this poses, the authors do a fantastic job describing the rationale of the study. Methods Was there a hypothesis beyond the research methods hypothesis that these patients were enrolled to answer? What were the patients consenting to? This might affect their willingness to give samples. A research study about research methods might enroll more poorly than a research study where the samples were tested for biomarkers, etc. An additional feasibility item might be whether the samples were adequate for analysis, though I recognize this is less novel. Inclusion, exclusion criteria: Please define “too unwell” if possible. You mention that if patients were discharged and readmitted, they were re-consented. While I suspect this is a small number, it may be informative to report this. It could speak to the ethics of continuing to obtain samples from a patient who is unable to withdraw consent. The discussion of surrogate consent is well-written. Typo on page 9, line 11. Missed period between “larger study[.] The socio…” Results Typo page 9 line 40. “58 patients approach[ed]”. Discussion Was there any feedback (qualitative, structured, or even quantitative) sought from participants and families? The discussion says that the patients and families found it acceptable. This is a key outcome as highlighted in the conclusion. I don’t suppose there was any prospective or structured evaluation of patient-family acceptability? Perhaps this aspect and most of the paragraph that begins page 12, line 6 are new results and belong in the result section? While a recap of key findings in the initial paragraph of the discussion section is desirable, these shouldn’t be new findings. In a feasibility study, acceptability, language used, and challenges are key results or even methods. In a similar vein, perhaps the authors could comment on the language used for consent (perhaps in the methods section). These insights will enhance the generalizability of these findings and relevance of this report.

	I would also cite the lack of a formal process by which to measure acceptability as a limitation of this study (if that is indeed the case). While there is probably no formal or validated survey instrument, I agree with the authors that this is a key aspect of successfully conducting research in this population. Overall, an interesting research report on methods in an important and understudied patient population. The authors provide good rationale for the study, and generally describe their methods and results well.

VERSION 1 – AUTHOR RESPONSE

Comments from Reviewer 1(RG)
1. 'Too many passive tenses and split infinitives ….'

Answer
Some changes have been made as suggested. We are happy with the format and text as written and do not perceive a problem in comprehension from our own internal peer review. However we are happy if the inhouse editorial team make editorial changes for the final publication.

Comments from Reviewer 2 (JH)

2. Comment
'fails to prove that collecting biological material from dying patients is feasible … only 10% of patients of all potential participants provided urine samples in the last weeks of life (if this is defined as in the month prior to death)'
and
'The greatest weakness of the study must be that only one-half of the participants provided samples in the last four weeks of life and that almost one third were alive more than 3 months after the study.'

We would disagree with these statements.
• We collected 52 samples from 11 patients (n=20) within the last 4 weeks of life. This was considered a success.
• For the first 6 weeks of sample collection an inclusion criteria was for the patient to have a urinary catheter in place. Thus 12 patients who had consented were not recruited. This requirement was removed for the latter 6 weeks of the study and allowed for greater recruitment.
• 44% of all potential patients did not have capacity and as such we legally could not approach these patients as we were bound by the Mental Capacity Act (UK) of 2005.
• The samples from patients alive more than 3 months after the study provided the important controls for the laboratory studies.
• We think it is appropriate to define dying patients as in the last month of life. We have some preliminary unpublished evidence suggestive that biochemical changes occur up to 3 weeks before death.

3. Comment
'Title. This is deceptive. This is not a paper describing the collection of biological material in the last weeks of life but about the collection of urine from patients with advanced disease.'
We would disagree with this statement.
• The Neuberger review outlined the difficulty of recognising when patients are dying. By its very nature collecting samples from dying patients is difficult. Thus collecting samples prospectively also

means that samples will be collected from those with advanced disease and who don't die.
• We collected 52 samples from 11 patients (n=20) within the last 4 weeks of life. We considered this a success.
• The process we outlined could be used for the collection of any biological material e.g. blood.

4. Comment
'What work if any has been done on the value of studying urine samples during the dying process. What specific molecular or metabolomics approach might provide useful information?'

Answer
Prior to this study there has never been any work which collected samples prospectively to investigate the process of dying or look for novel biomarkers. We have analysed our samples by GC-MS, with some initial PCR and western blotting studies. These results are unpublished. Our work is now supported by a Wellcome Trust seed award based on our initial findings.

5. Comment
'Methods. Were research staff employed specifically to carry out this project or was it unit staff collecting the samples?

Answer
No research staff were specifically employed to carry out this project. Patients were consented and samples collected by 2 palliative medicine specialist trainee doctors who were GCP trained (SC and AS). SC held a NIHR funded Academic Clinical Fellowship who co-wrote the ethics application and analysed the samples in the laboratory. They were not members of the treating team.

6. Comment
'Methods. The paper alludes to the difficulty of gatekeeping … yet this is an example of gate-keeping at the extreme. … this deserves further discussion.

We think that this is an unfair comment, the protocol had full ethical approval for this study. Also, in the EJC paper cited by the reviewer, she was involved in a study with palliative patients. In the methods, patients recruited 'had to be aware of the advanced state of their disease'. Our protocol, which discusses patients with the treating team aimed to achieve just that.

7. Comment
'Why was smoking status relevant?'
Answer
Smokers would have additional chemicals in their urine which we can detect by GC-MS analysis.

8. Comment
'issue of not-overburdening participants with lengthy information sheets … interesting to know what was in the 3 page information sheet'

Answer
As requested by the Research Ethics Committee the following topics were addressed; introduction to the study, why the study is being done, Why the patient has been asked to participate, what will happen if they agree to take part, Benefits to the patient, Risk to the patient, confidentiality issues (who will be looking at the records, that the information will be kept confidential), what will happen to the samples collected, Complaints or harm, who is organizing and funding the research, further information.

9. Comment
'What were the patients told about what their urine might be tested for?'

Answer
"We plan to analyse the urine of patients with advanced disease and how this changes towards the end of life. There is very little known about changes in a person's body at this incredibly important time. By doing this study we want to examine if the physical experiences of the patient can be seen in the genes and in the chemicals found in urine."
"The urine will be analysed for chemicals present. DNA will also be obtained, from which we will determine which genes are being expressed and those that are not."
The above 2 quotes come from the Patient Information Leaflet used.

10. Comment
'Could the PIL be included as an appendix?'

Answer
We are happy for it to be included. This is attached as a supplementary file.

11. Comment
'by agreeing to donate .. .actually contributing to a form of tissue bank. This raises a number of ethical issues. Did the participants consent to their urine being used for any non-specified test that might come along in the future?'

Answer
No. We specified in the research protocol how the samples would be analysed. The above question was addressed by the local Research Ethics Committee.

12. Comment
'Might this involve genetic testing in which case a separate consent form is often required'

Answer
No. The above question was addressed by the Research Ethics Committee.

13. Comment
'.. development of the consultee declaration process but query why this had to be repeated every time a urine sample was taken. This seems an unnecessary barrier to place around each collection.'

Answer
This was requested by the research ethics committee.

14. Comment
'The information in table 2 could be presented in the text'

Answer
We felt that in the interests of the articles word count and readability that the table provided more clarity.

15. Comment
'Discussion – the paper implies that palliative care patients form a particularly vulnerable population … I would suggest that this population is no more vulnerable that many other groups (for example

neonates and those in intensive care) ... there is now extensive literature showing how patients at the end of life are willing to be involved in research especially that is non-invasive. These points could be discussed further.'
and
'It is stated that the protocol used minimised stress to patients and their families. Worth highlighting increasing number of studies completed in this population … with interventions considerably more invasive.'

Answer
Palliative patients are a variable group with prognoses ranging from years to days or hours. Further, patients in a hospice usually have complex needs. This study was never about doing invasive research on 'palliative patients' – it was about the feasibility of collecting samples from patients towards the end of life. This cohort is an important and understudied population.
We have already written in the discussion 'that palliative patients want to participate in research'.

Comments from Reviewer 3 (NW)
All comments have been addressed by changes in the manuscript

VERSION 2 – REVIEW

REVIEWER	Nicholas Wysham Duke University, USA
REVIEW RETURNED	08-Aug-2016

GENERAL COMMENTS	I have no further requests. I believe the study is well conceived, well conducted, well described and makes an important contribution to the medical literature