**Author’s response to reviews**

**Title:** Case Report: A fatal case of disseminated adenovirus infection in a non-transplant adult haematology patient

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Reviewer reports/replies:

Maciej Przybylski (Reviewer 1):

There are several issues which have to be improved in the manuscript:

1. Manuscript is written in colloquial form. Writing style should be improved.
   - we are not sure what the reviewer means by this, as we have published multiple case reports previously using the same writing style. However, we have reviewed the text and made some amendments.

2. Lack of diagnostic methodology. There is no information about methods used and pathogens searched for (except HAdV and H. influenzae). In particular:
   - what method was used for HAdV DNA detection (regular or real-time PCR, in-house or commercially available, producer)?
   - the BAL testing for adenovirus and other respiratory viruses was performed using an in-house panel (which is described in the new paper shown below – which is now cited in the revised text).
   - the blood adenovirus PCR testing and quantitation was performed at a commercial laboratory (Micropathology Ltd., Coventry, UK: https://www.micropathology.com/), which has now been cited in the revised text (their methods are proprietary).
   - If BAL samples were tested with qualitative or quantitative PCR method for HAdV?
the BALs were only tested qualitatively using an in-house qualitative respiratory panel (as described in the publication below, which is now cited in the revised text):


- What was exact methodology of mycological laboratory diagnostics?

- these were just standard routine diagnostic laboratory diagnostic methods. We do not feel that including additional detail on these are useful or necessary as this would take up a lot of space distract from the focus of this case report is on the adenovirus infection.

- Was there testing for respiratory viruses other than HAdV?

- yes, and these were tested using an in-house panel of respiratory PCR tests, which have been described elsewhere – now cited in the revised text:


- How was adenovirus type determined?

- this was performed by AdV sequencing at the same commercial laboratory (Micropathology Ltd., Coventry, UK) – using their proprietary assay. This detail has now been added in the revised text

3. Conclusions presented by the Authors indicate the need for sequential diagnosis (first - detection of the virus the material corresponding with localized infection and then - blood testing). In such situations, however, today's consensus indicates detection of the virus in both materials at the same time, and in many clinical settings this approach is already routine for symptomatic patients from risk groups. Conclusions should be improved.

- clearly clinical practices will differ across different countries and institutions, depending on resources and funding available. Taking blood for a respiratory illness presentation is not routine practice here and may not be very cost-effective,
as most respiratory infections do not disseminate to cause generalised sepsis. But we have made some additional comments on this in the revised text.

Please, find manuscript file with comments.

- thank you. We have incorporated amendments (taking into account the comments from the other reviewers also) in the revised text.

Maureen O'Brien (Reviewer 2):

The authors present a case report of a patient treated for CLL who developed adenoviral pneumonitis followed by uncontrollable viremia which led to his death. They recommend that in the case of +adeno from BAL (or any localized site such as diarrhea), early evaluation of peripheral blood PCR for adenovirus is indicated which may allow earlier initiation of antiviral therapy.

Some additional information regarding this patient would be instructive for readers. What were serial absolute lymphocyte counts, T/B subsets, immunoglobulin levels throughout this patient's prolonged respiratory illnesses? intermittent values are provided but it would be helpful to have a comprehensive understanding of the degree of immune suppression experienced from his CLL therapy. Was his level of immune suppression considered expected given the therapy he received, or atypical? Is IVIG replacement for low IgG levels standard of practice for CLL patients with this level of immunosuppression or was it only given once the adenovirus was detected? Given the patient's progressive respiratory illness, why was cidofovir not initiated at the time of the positive BAL when he was diagnosed with adenoviral pneumonitis? Even when the peripheral blood PCR was positive one week later, adenovirus directed therapy was not initiated for another 4 days when the peripheral blood PCR dramatically rose.

- we don’t feel that presenting large tables of such serial laboratory data for one case is necessarily useful., however, some additional data on the immunoglobulin and CD4 cell count is now included. Also, note that these numbers will not tell us about the functionality of these white cells.

- IVIG is not given routinely to replace immunoglobulins in such patients, who had chemotherapy many months before the respiratory illness we are reporting developed. In this case IVIG was specifically given to treat the adenovirus infection, as it contains antibodies to various adenovirus types, depending on the exposure history of the various blood donors.

- the delay in monitoring for blood dissemination of HAdV and therefore potentially earlier treatment with cidofovir/systemic therapy for HAdV is the point of this report.

- with hindsight, perhaps we did not commence HAdV systemic monitoring and then cidofovir therapy early enough after the onset and confirmation of HAdV respiratory infection, but this is not routine practice yet here in the UK for non-transplant haematology patients.
cidofovir is a very nephrotoxic drug – so many transplant teams are reluctant to prescribe it empirically without firm evidence of need which may delay treatment leading to potentially poorer clinical outcomes – hence this need for this case report to stimulate some further discussion around this topic.

This case report would be most instructive if it included literature review and recommendations for immune surveillance and supportive care for CLL patients receiving fludarabine

- this has been expanded, as suggested.

Thomas Lion (Reviewer 3):

In the present manuscript Joffe et al. describe a case of pulmonary and systemic human adenovirus (HAdV) infection with fatal outcome upon multiorgan failure in an adult patient with CLL on chemotherapy. The authors suggest that immunocompromised patients displaying "peripheral" infection with HAdV should be tested for the virus at least once in peripheral blood in order to provide a rationale for early antiviral therapy in attempts to prevent adverse outcome. In general, the risk of life-threatening HAdV infections in immunocompromised adult patients may be an underestimated phenomenon and in this regard the present manuscript may raise the awareness of this potential problem. However, the conclusions drawn from the reported observations need to be reconsidered.

Specific comments

1. The decision to initiate anti-adenoviral treatment in the present case could have been based on the pulmonary symptoms and detection of HAdV in a BAL sample. If early initiation of treatment is important, as the authors claim in line with published data, what is the rationale for waiting until the detection of viremia before antiviral treatment is to be initiated?

- cidofovir is a very nephrotoxic drug and most cases of respiratory HAdV infection do not disseminate and are self-limiting without specific antiviral treatment. So laboratory confirmation of adenovirus viremia in the blood is important before commencing such a nephrotoxic drug.

2. The patient is reported to have experienced multiorgan failure which was attributed to invasive HAdV infection. Is this conclusion based merely on circumstantial evidence or was the organ affection demonstrated by detection of massively HAdV-infected organs post mortem?

- no additional post-mortem HAdV testing was performed so this was inferred from the high blood levels of HAdV DNA – which is not unreasonable in such a case
with such high levels of virus in the blood – other published case reports have also used high blood levels of HAdV as being correlated with disseminated HAdV disease

3. The authors suggest performing a single HAdV screening test in peripheral blood in immunosuppressed patients with "peripheral" infection with this virus in order to facilitate the detection of invasive infection. This approach is questionable even if one were to agree that the onset of treatment should be triggered by the detection of viremia (which is a question per se). Since the time point of viral invasion into peripheral blood is not predictable, a single blood test could reveal a negative result, depending on the selected time point of investigation. In this regard systematic monitoring might be more reasonable.

   - yes, we agree with the reviewer that a single sample may miss an AdV infection. We have amended this to serial (once or twice weekly) monitoring for AdV viremia for the duration of the AdV respiratory illness period.

4. What do the authors regard as a "peripheral" HAdV infection? Does a lung infection fit their definition?

   - this really refers to a non-blood sample, so a respiratory sample from the lung would fit this, as it is related to a single (respiratory/lung) compartment. In contrast, blood perfuses all body organs/systems. We have clarified this in the revised text.

Minor comment

The term HAdV "serotype" has been replaced by "type" because all HAdV recombinants discovered over the past ten years and accepted as novel types by the scientific community had been identified by molecular methods. The currently known number of HAdV types is far beyond 50.

   - thank you. We have rephrased this to ‘greater than 50 types’, as there seem to be 57 recognised types.

David A. Ornelles (Reviewer 4):

This report is succinct, informative and very well written. The only suggestion I might offer is to make explicit note of the absence of adenovirus in the first broncho-alveolar lavage. I assume this is the case since a H. flu was found and the sample "was negative for all other pathogens." If there was no data on the presence of adenovirus DNA in the first lavage, that should be noted.

   - thank you. Unfortunately the earlier (3/6/15) BAL was only tested for bacteria and fungi – not viruses. Only the later (11/8/15) BAL was tested for respiratory viruses. We have clarified this now in the revised text.
this is another learning point from this case report – that all 3 types of pathogens (bacteria, fungi, viruses) should be screened for in such haematological patients presenting with respiratory illness. This point has also been included in the revised text.