Author's response to reviews

Title: Cyclic neutropenia containing a novel gene mutation presenting with a necrotizing soft tissue infection and severe sepsis: A care report

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Author's response to reviews: see over
Answers to the reviewers’ comments

We wish to thank the reviewers for their careful reading of the manuscript. We have revised the manuscript according to their helpful recommendations.

To the Reviewer: Joan Robinson

Reviewer's report:
The authors report a single case of necrotizing fasciitis that seemed to respond to negative pressure wound therapy. The child was subsequently found to have cyclic neutropenia.

Major compulsory revisions

1. In the Case Presentation, please provide a few more details about the previous medical history. What type of respiratory infections was the child admitted for? How many admissions were there and how long were they? Did she have any outpatient infections that required antimicrobials?

   Comments were added concerning her previous medical history, including respiratory infections. She was hospitalized several times for pharyngitis and bronchitis. She was admitted twice with bronchiolitis, and once with pharyngitis. She visited the emergency department for high fever with pharyngitis once. She stayed in the hospital an average of 5–6 days per visit. At the time of admission, no antimicrobials had been administered outside the hospital setting.

2. What do you mean by “idiopathic cyclic neutropenic events”?

   During the cyclic pattern of neutropenic events seen in this patient, the parents refused further evaluation, so the cause was unknown. To prevent confusion, we changed the term “idiopathic cyclic neutropenic events” to “unusual neutropenic events.”

3. Was the child neutropenic when she presented this time? What were her vital signs (I know that you said she was stable, but I find it hard to believe that they were normal)? Was she in pain? Was her level of consciousness normal?

   Her initial vital signs were stable (BP 90/60 mm Hg, heart rate 100 bpm), and she had a body temperature of 37.6°C. She complained of pain in the axillary area, and her level of consciousness was alert. She appeared dehydrated, and her affect was slightly subdued. She was hospitalized, and antibiotics and fluid therapy were begun to treat suspected cellulitis. Within 8 hours, she deteriorated rapidly, requiring inotropic support and intubation for the management of respiratory failure. We were also surprised to see the unusually rapid progression of sepsis at this time. However, this rapid onset of sepsis is typical in necrotizing fasciitis (soft tissue infection), which is one of the reasons for prompt diagnosis.
and treatment of such cases.

4. What antibiotics were started? What antibiotics were used in the PICU after debridement? What were the susceptibilities of the Pseudomonas? How long did the bacteremia persist? How long did the neutropenia persist? What day did she come off inotropes? When did the daily surgical debridements end (presumably the day that you started the negative pressure wound therapy but his needs to be clarified).

Initial antibiotics were piperacillin plus tazobactam. After debridement, we changed the antibiotics to cefepime and imipenem. No organisms were cultured from the blood 2 days after the initiation of the initial antibiotics. She required inotropic agents to maintain blood pressure for 5 days until we applied negative pressure wound therapy (NPWT). After this effective source control, we tapered the dose of the inotropics. The initial neutrophil count was 329 cells/µl (WBC count was 3,400/µl). Neutropenia persisted for only 2 days after an injection of G-CSF (the neutrophil count normalized to 4,300/µl and the WBC normalized to 10,600/µl three days after admission). We began daily surgical debridement at the time of diagnosis, changing to NPWP 5 days after the initial surgical treatment. We believe that her symptoms rapidly improved after adequate infection source control. The isolated *Pseudomonas* was resistant to ampicillin and cephalosporin, but susceptible to imipenem. *Staphylococcus hominis* was resistant to beta-lactam antibiotics but susceptible to vancomycin. We changed the antibiotics to vancomycin and imipenem after we obtained an antibiotics sensitivity report for these organisms.

5. In talking about her neutropenia, what do you mean by “the same events had also been detected in previous medical records.” I ask as you implied earlier that the parents refused investigations for cyclic neutropenia.

I removed the redundant text.

6. At what point during the admission did you figure out that the child’s mother had cyclic neutropenia?

We obtained the mother’s own frequent infection history when we took the child’s history from her mother. The mother hesitated to expose her whole previous history, likely because of the cultural stigma of discussing genetic diseases and traits.

7. I do not understand the phrase “and through on-line in silico functional prediction analysis, the variation was predicted as a probable cause of deteriorating protein function” and suspect that many other readers will not understand it either.

We corrected this phrase in the manuscript as follows:

“Since sequence variations are not known as mutations, we performed two virtual protein function analyses to predict whether the amino acid substitution found might affect protein function. Using the PolyPhen analysis tool, the variation was predicted as ‘probably damaging’ to protein function; using the SIFT tool, the result was ‘Damaging’ protein function. Therefore, the p.G125W missense variant of the ELANE gene may negatively
affect neutrophil elastase function, which plays a pathophysiological role in CN.

8. Although surgical debridement is the preferred therapy for necrotizing fasciitis, I think that it is out-of-date to call it “mandatory”. There are now many case reports of patients surviving despite limited or no debridement, typically because the location or extent of the necrotizing fasciitis makes full debridement impractical.

We don’t agree with your opinion that surgical debridement for necrotizing soft tissue infection is not mandatory. Necrotizing soft tissue infection, including necrotizing fasciitis, is a highly lethal disease. The mainstay of treatment is early and complete surgical debridement, combined with antimicrobial therapy, close monitoring, and physiological support. Novel therapeutic strategies, including hyperbaric oxygen and intravenous immunoglobulin, have been described, but their effects are controversial. Even for the more simple soft tissue infections and abscesses, adequate control of the source of infection critically improves the outcome. However, as you indicated, frequent surgical treatments are burdens for pediatric patients. This is the reason why NPWP is an effective treatment modality in such cases. We changed the term “mandatory” to the less aggressive term “necessary.”

9. I am curious about previous reports of use of negative pressure wound therapy for necrotizing fasciitis and would like more detail. The reader is referred only to reference 9 (which is not about necrotizing fasciitis) but references 7 and 8 also mention previous cases. Telling the reader what organisms were involved is relevant as it seems possible that the efficacy of negative pressure wound therapy would be related in some way to the pathogen. A table combining this with previous cases might be helpful if there are 5 or more cases.

We strongly agree with your recommendation to illustrate other previous reports and show these tables for readers. However, the other reviewer recommended that we not place undue emphasis on this less certain effect of NPWT because the point of this case report is CN with a novel mutation. Therefore, I changed the title (“Cyclic neutropenia mutation with necrotizing soft tissue infection and severe sepsis: A case study”) and revised the manuscript from this perspective.

10. One of the limitations of this report is that you cannot be certain that the negative pressure wound therapy resulted in the child’s clinical improvement. In particular, you have not convinced me that she received optimal antimicrobials. I would also argue that perhaps she got better as she got more neutrophils but you have not given me any information about this. The discussion should be much more tentative about the potential benefit of negative pressure wound therapy for necrotizing fasciitis given the current level of evidence.

We feel that NPWT effectively controls the patient’s wound because she had not improved prior to its administration, despite seemingly adequate treatment. However, this is only a case report to obtain sufficient evidence of an effect, but additional cases are necessary to confirm these conclusions. Thus, we decided not to focus on NPWT in this case report. As for the antimicrobials, when the antibiotics were first administered, the selection of
antibiotics and the physiological support therapy were adequate. I described this in detail above and added it to the manuscript.

Minor essential revisions
1. The abstract could be shorter. Some points are mentioned more than once.
   We revised the abstract as you recommended.

2. Abstract - Please correct: Despite daily wound debridement and irrigation, sepsis was not controlled and fasciitis spread out her chest wall and neck area.
   This point was corrected.

3. Background – “mouth ulcer” should be plural
   This point was revised.

4. Background – “if not early diagnosed” needs to be re-worded
   This point was revised.

5. I will not comment further on minor wording changes that should be done as there are too many. The authors should ask a colleague with excellent English skills to review the paper for them. I could always understand their meaning but often the wording is awkward or not grammatically correct.
   We had the revised manuscript reviewed by Bioscience Writers, LLC, a company that specializes in high-quality scientific writing and editing services.

6. Many of the paragraphs are very long. The paper would be easier to read if they were shorter and more concise. The discussion could be shortened by about one-third without losing any of the important concepts in the paper.

   Discretionary revisions
   This point was corrected.

1. Pseudomonas is an unusual cause of necrotizing fasciitis. I would mention somewhere about the fact that invasive infection with this organism was another clue to the diagnosis of cyclic neutropenia.
   The exact invasive mechanism of this unusual pathogen is unclear. We infer that this organism might have originated from the colonization of her skin during a previous hospitalization event. By compromising her local defenses, this bacterium could proliferate rapidly, causing the destructive infection that led to septic shock. We added this comment to the discussion.
To the Reviewer: Michael Hawkes

Reviewer's report:
The authors are to be congratulated on an interesting case report where a molecular diagnosis of ELANE leukocyte elastase mutation was associated with cyclic neutropenia, the likely predisposing factor for an aggressive soft tissue infection and Pseudomonas aeruginosa bacteremia.
The major strength of the report is the molecular diagnosis, and description of a new point mutation in the ELANE gene, present in both mother and daughter with similar neutropenia phenotype (autosomal dominant inheritance).

(Discretionary revision)
I am less impressed with the "wound vac" aspect of the case, which may have accelerated healing, or may have been only temporally associated with the healing expected with surgical debridement and (presumably) appropriate antibiotic therapy. This anecdotal report cannot establish with any certainty the benefit of negative pressure dressings in the management of necrotising fasciitis. I wonder if this aspect should be given less importance in the case report (do not include it in the title).

We changed the title to “Cyclic neutropenia containing a novel gene mutation presenting with a necrotizing soft tissue infection and severe sepsis: A case report”

(Discretionary revision)
The authors consistently use the term necrotizing fasciitis throughout the text which is probably an accurate pathologic description (but cellulitis and myositis also seem to be present). The microbiology is a bit different from the usual pattern of nec fasc (usually pure culture of group A strep, or polymicrobial necrotising gangrene with anaerobes). The initial lesion (knowing the microbiological etiology) is reminiscent of "ecthyma gangrenosum". "Deep soft tissue infection" might be another less leading description of the infection (rather than nec fasc).

As you recommended, we changed the term “necrotizing fasciitis” to “necrotizing soft tissue infection.”

Where did the Pseudomonas come from? We generally think of this as a nosocomial pathogen - it would seem there was a history of recurrent hospitalizations for pneumonia. Comment on the microbiology in the discussion would be helpful.

The exact invasive mechanism of this unusual pathogen is unclear. We infer that this organism might have originated from the colonization of her skin during a previous
hospitalization event. By compromising her local defenses, this bacterium could proliferate rapidly, causing the destructive infection that led to septic shock. We added this comment to the discussion.

(Minor essential revisions)

1. Please report the antibiotic susceptibility pattern of the Pseudomonas isolate and the antibiotics used to treat the deep soft tissue infection and bacteremia.

Initial antibiotics were piperacillin plus tazobactam. After debridement, we changed the antibiotics to cefepime and imipenem. The isolated *Pseudomonas* was resistant to ampicillin and cephalosporin, but susceptible to imipenem. *Staphylococcus hominis* was resistant to beta-lactam antibiotics but susceptible to vancomycin. We changed the antibiotics to vancomycin and imipenem after we obtained an antibiotics sensitivity report for these organisms.

2. Please provide the method used for species-level identification of the (coagulase-negative) *Staphylococcus hominis*, antibiotic susceptibility and whether the authors considered this a causative pathogen or a skin commensal.

*Staphylococcus hominis* was resistant to beta-lactam antibiotics and susceptible to vancomycin. We changed the antibiotics to vancomycin and imipenem after we obtained an antibiotics sensitivity report for these organisms. However, a subsequent culture of the wound failed to isolate this organism. We thus concluded that it was not the causative pathogen for the necrotizing infection.

3. ELANE mutations are thought to lead to a "gain-of-function" in the neutrophil granule protein leukocyte elastase (aberrant processing, packaging), with consequent cellular toxicity to neutrophil precursors. The point mutation (c.373G>T, p.Gly125Trp) in exon 4 appeared to confer "deteriorating protein function" according to an in silico analysis that was not well described. Can the authors more clearly relate the mutation (seen in both mother and daughter with similar CN phenotype) to the protein structure and speculate on the basis of the neutropenia? E.g., missense mutation in the terminal peptide that confers protection to cellular elements during protein synthesis, or other. I think it would be valuable to add this newly described point mutation to a database such as the Human Gene Mutation Database.

With regard to your kind comments and another reviewer’s remarks, we corrected the last phrase in the Case presentation section in the manuscript, as follows:

“Since sequence variations are not known as mutations, we performed two virtual protein function analyses to predict whether the amino acid substitution found might affect protein function. Using the PolyPhen analysis tool, the variation was predicted as ‘probably damaging’ to protein function; using the SIFT tool, the result was ‘Damaging’ protein function. Therefore, the p.G125W missense variant of the ELANE gene may negatively affect neutrophil elastase function, which plays a pathophysiological role in CN.”

*According to an article by Horwitz, et al. (reference 10), the pathophysiological role of the
ELANE gene mutation in CN is not yet fully understood. Therefore, we regretfully cannot relate this mutation to the pathogenesis of neutropenia. As you mentioned, we are planning to register this point mutation after acceptance of this paper to established mutation databases, such as HGMD, ClinVAR, etc.

4. Please provide the serial neutrophil counts in hospital that demonstrated the cyclic pattern of the neutropenia. Providing detail for this component of the phenotype will be convincing for CN (rather than an incompletely documented, suspected CN). The phenotype should be well described for a clear association with the genetic mutation in ELANE gene.

The initial neutrophil count was 329 cells/µl, and the white blood cell count was 3,400/µl on admission. Neutropenia persisted for only 2 days after an injection of G-CSF (the neutrophil count normalized to 4,300/µl and the WBC normalized to 10,600/µl three days after admission). However, when we reviewed the serial measurements of absolute neutrophil counts for the period of hospitalizations, including laboratory data from the outpatient department, we found neutrophil count oscillations with a 21-day periodicity and neutropenia at the nadir of the cycle.

(Minor essential revisions)

Editorial remarks:
"very rare", "extremely rare" are used throughout the text for conditions that we do see from time to time in clinical practice. I think "rare" suffices
CN appears in abstract without prior definition of the abbreviation
fasciitis "on" the axilla - wording awkward - fasciitis of the axillary tissues...?

We revised these phrases as you recommended.
We define the term cyclic neutropenia (CN) at its first use and included it in the paragraph of Abbreviations.

bottom of page 2: suspicion of congenital neutropenia
"under the diagnosis" (p3) awkward
p5: angiogenesis
other awkward sentences: have another native English speaker read through for grammar/sentence structure

We revised these phrases as you recommended.

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