A case report of cyclic thrombocytopenia with statistically significant neutrophil oscillations

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Abstract

Background: Cyclic thrombocytopenia (CT) is a hematological condition characterized by severe thrombocytopenia alternating with thrombocytosis. Understanding the features of platelet count oscillations, the involvement of other cell lines, and response to thrombopoietin (TPO) stimulation will help clinicians better manage this challenging disorder.

Case presentation: We describe the clinical and laboratory features of four patients with cyclical thrombocytopenia. We used Lomb-Scargle periodogram analysis to determine the existence of statistically significant periodicity of platelet count oscillations and to investigate possible cycling in other cell lines. One patient was found to have concomitant, statistically significant neutrophil cycling with the same period as the platelets, a feature that to our knowledge has never been reported before in the literature on CT. We also describe platelet count responses to treatment with TPO receptor agonists in three patients.

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**Results:** There were statistically significant ($p \leq 0.05$) oscillations in platelet counts with periods of 39, 23, 28 and 42 days for patients A, B, C and D respectively. Patient A also had statistically significant oscillations in neutrophil count at the same period (39 days) as in the platelet count. Cycling platelet patterns were independent of treatment with corticosteroids, intravenous immunoglobulin or danazol treatment. TPO receptor agonists were used to treat severe thrombocytopenia in patients A, B and C: For patients A and B, TPO receptor agonist treatment caused extreme thrombocytosis. For patient C, the thrombocytopenia improved after low doses of TPO receptor agonist medication.

**Conclusion:** The concomitant platelet and neutrophil oscillations in patient A, coupled with no indication of an ELANE mutation characteristic of cyclical neutropenia, suggests that CT may represent an acquired stem cell defect with concomitant cycling in other cell lines. Additionally, our report highlights that TPO receptor agonists should be used with caution in patients with pre-existing CT because it can cause extreme thrombocytosis.

**Background**

Cyclic thrombocytopenia (CT) is a rare blood disorder characterized by periodic cycling in platelet counts. Thrombocytopenia and thrombocytosis can be extreme, resulting in an increased risk of bleeding alternating with an increased risk of thrombosis. The mechanisms that lead to CT may include anti-platelet antibodies, suggesting an autoimmune component with cyclical destruction, or abnormal feedback regulating platelet production or both [1]. Treatment of CT is challenging and the safety of thrombopoietin (TPO) receptor agonist medications is uncertain. Understanding the clinical and laboratory features of platelet count oscillations, the involvement of other cell lines, and response to thrombopoietin stimulation will help clinicians manage this challenging disorder.

The usual way of assessing the existence of periodicity in a data series is the Fourier power spectrum, which is useful when data are evenly sampled. However, the technique is notoriously unreliable when data are unequally sampled as is usually the case for blood cell counts. This problem exists in many fields, including astrophysics, and Lomb [2] developed an alternative which is an extension and refinement of the Fourier spectral technique, now known as the Lomb periodogram. Scargle [3] placed this technique on a solid foundation by establishing the statistical significance, or $p$-value, of any peak in the Lomb periodogram and this is the technique (now known as Lomb-Scargle periodogram analysis) that we employed. This technique is described in [4] and has been implemented at the web site http://cyclicneutropenia.org/.
Case reports

We describe four patients with CT, one of whom was found to have concomitant, statistically significant neutrophil cycling with the same period as the platelets, a feature that to our knowledge is unreported in the literature on CT. Three patients received TPO receptor agonists: treatment was discontinued in two patients because of worsening fluctuations in platelet counts.

**Patient A:** A 49 year old male presented to hospital in May 1998 because of spontaneous bruising and mucosal bleeding. His platelet count was $2 \times 10^9$/L. The other blood counts were normal, and no other laboratory abnormalities were noted. He had a history of alopecia totalis, but no other concomitant illness and no family history of blood disorders. He was treated with prednisone (100 mg daily) and his platelet count improved, but when the dose of prednisone was gradually reduced and stopped, the thrombocytopenia returned. He subsequently underwent laparoscopic splenectomy in December 1998, which resulted in a positive platelet count response that lasted four years. In April 2003, Idiopathic thrombocytopenic purpura (ITP) relapsed and after another course of prednisone, he developed a pronounced cyclical pattern of thrombocytopenia (Figure 1A), with platelet count levels fluctuating with a statistically significant ($p \leq 10^{-22}$) period of 39 days (Figure 1B) from nadir values of less than $5 \times 10^9$/L to peak values of greater than $900 \times 10^9$/L. Statistically significant ($p \leq 0.001$, Figure 1D) oscillations of exactly the same period in neutrophil counts (Figure 1C) were also found, but the neutrophil nadir never dropped below the normal range.

Cyclic thrombocytopenia persisted for over 10 years despite treatment with corticosteroids, intravenous immunoglobulin, danazol, pulse dexamethasone, and rituximab (4 weekly doses 375 mg/m²). TPO levels were measured serially for a period of 6 weeks (see Figure 1E): TPO levels were undetectable during periods of extreme thrombocytosis, and increased when platelet counts were low.

Treatment with the oral TPO receptor agonist eltrombopag was started and timed with anticipated periods of thrombocytopenia. Patient A received 50 mg of eltrombopag daily from 30 November to 6 December 2010, 2 January to 15 January 2011, and 22 February to 14 March 2011. Treatment, however, resulted in extreme thrombocytosis and did not alter the cycle pattern or period (Figure 1F, periods of treatment within the double arrows), and eltrombopag was discontinued thereafter.

**Patient B:** A 53 year old male presented with severe ITP in 2009. Splenectomy was carried out October 1999, but he continued to have severe thrombocytopenia requiring frequent doses of intravenous immunoglobulin (IVIG) and corticosteroids. In January 2001 he was started on a combination of immunosuppressant medications, which included azathioprine,
Figure 1: Patient A data, with time measured in days since diagnosis. (A) Platelet count. (B) Platelet power spectrum. (C) Neutrophil count. (D) Neutrophil power spectrum. (E) Platelet count and TPO levels over one cycle. (F) Platelet count for three cycles during three periods of treatment with eltrombopag (demarcated by dashed lines and double arrows).

cyclosporine and mycophenolate [5]. In April 2003, the platelet count levels began to oscillate periodically between a nadir of $< 10 \times 10^9$/L and a peak of 300400 $\times 10^9$/L (Figure 2A). During episodes of severe thrombocytopenia, he frequently had bleeding with oral mucosal blood blisters. Treatment with danazol was added but had no effect. In January 2009 eltrombopag was started. This resulted in extreme thrombocytosis (peak platelet count $1,361 \times 10^9$/L) and eltrombopag and all immunosuppressant medications were stopped. Following that, the patient experienced a period of severe thrombocytopenia (platelets $< 10 \times 10^9$/L) for approximately 4 weeks. Subsequently, eltrombopag was slowly restarted and immunosuppressant medications were re-introduced. The cyclical thrombocytopenia became less severe with higher nadir platelet values and 4 years later the cyclical pattern resolved. At the last follow-up in May 2015, the patients medications were eltrombopag 75 mg daily, and low doses of azathioprine and mycophenolate. The patient’s neutrophil count was normal, and despite apparent fluctuations, statistically significant cyclicity in the neutrophil count was not detected (Figure 2B).

**Patient C:** A 40 year old male who presented with severe thrombocytopenia in 1997 and required splenectomy for refractory ITP in 1998. He remained in remission for 11 years until he presented again with severe thrombocytopenia (platelet count of $4 \times 10^9$/L) and bleeding in 2009. A pattern of cyclical platelet count oscillations became apparent (Figure
He received prednisone and IVIG with an initial platelet count response, but ongoing treatment was required because of recurrent thrombocytopenia. Weekly treatment with subcutaneous romiplostim (a TPO receptor agonist) was started. Low doses of romiplostim produced a good platelet count response (initially 1 mcg/kg per week, then 0.5 mcg/kg per week). The cyclical pattern continued with platelet count peaks to $330 \times 10^9/L$, nadirs of $30 \times 10^9/L$ and a period of 28 days ($p = 0.05$). Romiplostim was stopped 15 months later because platelet count nadirs remained above $100 \times 10^9/L$ and the cyclical pattern of thrombocytopenia became less pronounced. The patients neutrophil count was low and cyclicity in the neutrophil count was not detected (Figure 2D).

**Patient D**: A 47 year old female who presented with severe thrombocytopenia due to ITP in 1996. Platelet counts improved after treatment with three cycles of high dose dexamethasone, but soon thereafter she developed a pattern of platelet count cycling (period of 42 days, $p \leq 1.5 \times 10^{-14}$) resulting in periods of severe thrombocytopenia associated with petechiae, oral mucous membrane blood blisters and nosebleeds (Figure 2E). Treatment with combination immunosuppressive therapy [5] resulted in a complete and sustained response for two years off therapy; however, subsequently, severe cyclic thrombocytopenia recurred and persisted despite reinstitution of immunosuppressant medications. The neutrophil count was generally low, and no cyclicity in the neutrophil count was detected (Figure 2F).

Figure 2: Platelet and neutrophil data for the other three patients, with time measured in days since diagnosis. (A) Platelets and (B) neutrophils for patient B, (C) Platelets and (D) neutrophils for patient C, and (E) Platelets and (F) neutrophils for patient D.
Statistical evaluation of cyclical counts

We tested the platelet, neutrophil, monocyte, erythrocyte, basophil and eosinophil counts for statistically significant periodicity in patient A using Lomb-Scargle periodogram analysis [2, 3]. We restricted the analysis to a 26-month period (30 April 2003 to 23 June 2005) that contained the data with the most frequent sampling. Cell counts and power spectra are shown in Figure 1 for platelets (panels A and B) and neutrophils (panels C and D). The analysis indicates statistically significant cycling at a period of 39 days in both platelets \( (p \leq 10^{-22}) \) and neutrophils \( (p \leq 0.001) \). Monocytes also showed cycling with the same period (data not shown). There was no evidence for any statistically significant cycling of erythrocytes, basophils, or eosinophils. The cycling of neutrophils was closely entrained with the platelet counts, with the peak of neutrophils preceding the peak of platelets by 8.3 ± 2.0 days. We sequenced the elastase gene for mutations previously reported in cyclic neutropenia [6]; no mutations were found. Thus the neutrophil oscillations in patient A are not due to cyclical neutropenia.

The results of the analysis for all patients is contained in the following table.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Platelet period (days)</th>
<th>p-value</th>
<th>Neutrophil period (days)</th>
<th>p-value</th>
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<td>(10^{-22})</td>
<td>39</td>
<td>(10^{-3})</td>
</tr>
<tr>
<td>B</td>
<td>23</td>
<td>0.05</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>28</td>
<td>0.01</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>D</td>
<td>42</td>
<td>(1.5 \times 10^{-14})</td>
<td>None</td>
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</tr>
</tbody>
</table>

Discussion

Cyclic thrombocytopenia is a hematological disorder that has serious implications for bleeding and thrombosis risk for patients. It is difficult to treat since conventional ITP treatments are rarely successful. In this case series we applied statistical analysis to characterize the periodicity of platelet count fluctuations and report our experience using TPO receptor agonists to treat this condition. We found that platelet count fluctuations are regular and predictable and that one patient had concomitant, statistically significant cycling in neutrophils. Treatment with TPO-based therapies should be used with caution because they can worsen the thrombocytosis.

Patient A had features of classic CT, but with associated cyclical neutropenia. The appearance of synchronous cycling of neutrophils with close entrainment of the two cycles suggests a dysregulation involving a common hematopoietic progenitor cell and a shared signaling pathway. The initial response to corticosteroids suggests an autoimmune basis to
the original presentation; however, the immune target may be early progenitor cells in the
bone marrow affecting precursors of megakaryocytes and granulocytes, rather than platelets
in circulation. The synchrony of the peaks suggests that TPO may be driving the process.
This is consistent with our observations of fluctuating TPO levels in this patient, as has
been shown by others [7]. TPO receptors are present on hematopoietic stem cells, which
may explain how both platelets and neutrophils were affected. Platelet cycling has previously
been reported in patients with predominant cyclic neutropenia, which may be due to the
periodic interruptions of neutrophil production and fluctuations of endogenous Granulocyte-
 colony stimulating factor (G-CSF) and other cytokines [8]. Similarly, concomitant cycling of
neutrophils has been previously claimed in a patient with pronounced CT [9] but when we
tested the neutrophil data in [9] there was no evidence of a statistically significant oscillation.
A preliminary report [10] of gene expression profile of two male patients with CT published
in abstract form noted cyclic changes in interferon responsive genes as well as transcription
factors controlling lineage differentiation. Cytokine oscillations have also been documented
in periodic hematological diseases, including CT [9, 11–13].

Cyclic neutropenia is usually an inherited genetic disorder with onset in early childhood
and associated with failure to maintain the myeloid progenitor compartment. Mutations in
the gene for neutrophil elastase cause production of an abnormal enzyme that is probably
misfolded, thereby triggering apoptosis in the progenitor cells causing severe neutropenia.
The rapid recovery of neutrophils is likely due to a surge in G-CSF and other cytokines in
response to the neutropenia. Patient A did not have the characteristic features of cyclic
neutropenia or mutations in ELANE gene characteristic of the heritable form of this disease.

Thrombopoietin receptor agonists are a new class of medications used to treat patients
with chronic ITP. Patients A, B and C in our series were treated with these agents. Patient A
developed extreme thrombocytosis after treatment with eltrombopag even when doses were
timed to coincide with anticipated periods of thrombocytopenia. Patient B also developed
extreme thrombocytosis after receiving eltrombopag, followed by severe thrombocytopenia
once the eltrombopag was stopped. Once eltrombopag and immunosuppressant medications
were reintroduced gradually, the platelet count nadirs improved. Finally, patient C developed
a subtle cyclical thrombocytopenia after treatment with romiplostim. These agents must be
used with caution in patients with cyclical thrombocytopenia since they can cause extreme
thrombocytosis. This occurrence can lead to the abrupt discontinuation of the medication,
which will exacerbate rebound thrombocytopenia [14].

In the past it was thought that a feature of cyclical thrombocytopenia was that the fluc-
tuations appear only in the platelets and not in the white or red blood cells. There have
been two relatively extensive surveys of the literature on cyclical thrombocytopenia [7, 15]
that analyzed well-documented cases of platelet fluctuations. In no case was there a report of fluctuations in the red or white blood cells. In other existing cyclical hematological disorders like cyclical neutropenia [16] or periodic chronic myelogenous leukemia [17], fluctuations can appear in all major blood cell lines and are all at the same period in a given subject. These diseases are believed to arise from the interaction between the hematopoietic stem cell compartment and peripheral control mechanisms. However, because in CT fluctuations had been observed only in the platelets, a destabilization of a peripheral control mechanism was postulated to play an important role in the genesis of this disorder [7, 18]. Though the control of neutrophil production as well as the regulation of erythropoiesis have been the subject of numerous modeling studies, there have been fewer treating the regulation of platelets production. One of the earliest was that of Wichmann et al. [19] which was followed by an exposition of their complete model for hematopoiesis [20]. Von Schulthess et al. [21], noting the existence of oscillations in the platelet counts of normal humans, put forward a conceptually different model for thrombopoiesis, which was followed by Bélair and Mackey [22]. Building on this work, Santillan et al. [18] refined the model attempting to understand cyclical thrombocytopenia, and this was subsequently modified by Apostu and Mackey [7] and Langlois et al. [23], again to understand the origins of cyclical thrombocytopenia. The latter modeling work has suggested that a defective TPO-TPO receptor interaction may contribute to the pathogenesis. Recently, Zhang et al. [24] described an interesting case of CT due to a heterozygous loss-of-function mutation of TPO receptor, further suggesting that CT may be due to abnormalities with the TPO receptor. It is fair to say, however, that these previous modeling attempts to understand the mechanistic origins of cyclical thrombocytopenia are not capable of fully explaining the observations of statistically significant oscillations with identical periods in both platelets and neutrophils of patient A.

Our case series is unique because we applied statistical analysis to a large amount of patient data to describe the periods of platelet and neutrophil oscillations, found that one patient had statistically significant concomitant cycling in neutrophils with the same period as in the platelets, a finding which, to our knowledge, has never been reported before in a case of CT, and because we discussed the hazards of using TPO receptor agonists to treat this CT. Neutrophil fluctuations may be underreported because fluctuations in neutrophil count levels do not give rise to symptoms, and because neutrophil levels, even at the nadir, may remain within the normal range.
List of abbreviations

CT – Cyclic Thrombocytopenia, TPO – Thrombopoietin, ITP – Idiopathic thrombocytopenic purpura, IVIG – intravenous immunoglobulin, C-CSF – Granulocyte-colony stimulating factor.

Ethics approval and consent to participate

The study was approved by the Hamilton Integrated Research Ethics Board (REB-Number: 10-499-C). Research ethics board approval forms are available on request.

Consent for publication

We did not report any personal information and all data was anonymized. A retrospective chart review with ethics approval from the Hamilton Integrated Research Ethics Board is available upon reasonable request.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

B. Leber reports that he is a member of the Medical Advisory Board of Novartis Canada. D. M. Arnold reports personal fees from Amgen (Advisory board member for Nplate (romiplostim)), grants from GlaxoSmithKline (Investigator Sponsored study in immune thrombocytopenia (ITP)), and personal fees from Bristol Myers Squibb (Consultant for research program in ITP), outside of the submitted work.

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Author’s contributions

G. P. Langlois collected a portion of the patient data, contributed to the LS periodogram analysis of the patient data, prepared the figures, interpreted the results of the LS periodogram, wrote and edited some of the text, and prepared the final version of the manuscript. J. Potts initiated the collaboration of this project by contacting M. C. Mackey, initiated data collection, identified patient treatment through reviewing clinical records, initiated patient testing cyclic neutropenia (ELANE Analysis), and was one of the two attending doctors to the patient. B. Leber interpreted the results, wrote and edited some of the text, and was one of the two attending doctors to the patient. D. C. Dale interpreted the results, was responsible for the ELANE analysis for cyclical neutropenia, wrote and edited some the text. D. M. Arnold contributed the patient data, supervised the chart review, interpreted the results, edited the text and approved the final version of the manuscript. M. C. Mackey initiated the collaboration at the request of J. Potts, contributed to the Lomb-Scargle periodogram analysis of the patient data, wrote and edited some of the text, and approved the final version of the manuscript.

References


